Characteristics of Recovery From the Euthyroid Sick Syndrome Induced by Tumor Necrosis Factor Alpha in Cancer Patients

R.A. Feelders, A.J.G. Swaak, J.A. Romijn, A.M.M. Eggermont, E.T. Tielens, G. Vreugdenhil, E. Endert, H.G. van Eijk, and A. Berghout

Cytokines have been implicated in the pathogenesis of the euthyroid sick syndrome. Isolated limb perfusion (ILP) with recombinant human tumor necrosis factor alpha (rTNF) and melphalan in patients with melanoma or sarcoma is accompanied by high systemic TNF levels. We examined the prolonged effects (7 days) of ILP on thyroid hormone metabolism with respect to induction and recovery of the euthyroid sick syndrome in six cancer patients. After ILP, when the limb is reconnected to the systemic circulation, leakage of residual rTNF resulted in systemic peak levels at 10 minutes postperfusion followed by a parallel increase in plasma interleukin-6 (IL-6) and cortisol, with maximum levels at 4 hours ($P < .05$). A rapid decrease was observed at 5 minutes for plasma triiodothyronine (T3), reverse T3 (rT3), thyroxine (T4), and thyroxine-binding globulin (TBG) ($P < .05$), whereas free T4 (FT4) and T3-uptake showed a sharp increase, with peak levels at 5 minutes ($P < .05$). T3, T4, and TBG levels remained low until 24 hours after ILP. In contrast, rT3 increased above pretreatment values to maximum levels at 24 hours ($P < .05$). Plasma thyrotropin (TSH) showed an initial decrease at 4 hours postperfusion ($P < .05$) but exceeded pretreatment values from day 1 to day 7 (by +94% ± 43% to +155% ± 66%, $P < .05$), preceding the recovery of T4 and T3 levels. T3 and rT3 returned to initial values at day 4. T4 and TBG levels recovered at day 2. T4 exceeded basal values at days 5 to 7 ($P < .05$). It is concluded that ILP with rTNF induces a euthyroid sick syndrome either directly or indirectly through other mediators such as IL-6 or cortisol. The recovery from this euthyroid sick syndrome is, at least in part, TSH-dependent, since the prolonged elevation of TSH values preceded and persisted during the normalization of T3 and the elevation of T4 levels. This biphasic pattern of induction of and recovery from the euthyroid sick syndrome may be a general feature of nonthyroidal disease. The euthyroid sick syndrome should be interpreted not only in relation to the presence of nonthyroidal diseases but also in relation to the recovery from these diseases.

NONTHYROIDAL ILLNESS is frequently associated with profound changes in thyroid hormone metabolism, referred to as the euthyroid sick syndrome.[1,2] This syndrome is characterized by low plasma triiodothyronine (T3) concentrations and increased levels of reverse T3 (rT3).[1,2] Thyroxine (T4) levels may be within the normal range, but decrease with increasing severity of the underlying disease.[3] Thyrotropin (TSH) concentrations are usually normal, although slightly elevated or reduced TSH levels can also be found.[4,5] The recovery from the euthyroid sick syndrome has not been studied prospectively. In surviving patients with a variety of critical illnesses and severe hypothyroxinemia (T4 < 35 nmol/L), Hamblin et al[6] observed an increase in TSH and T4 during the recovery phase. These results suggest that the recovery from the euthyroid sick syndrome may also have typical characteristics, at least in the presence of hypothyroxinemia.

The alterations in thyroid hormones in nonthyroidal illness are remarkably similar despite the heterogeneity of the underlying etiology. Therefore, it has been suggested that final common pathways are involved in the pathogenesis of the euthyroid sick syndrome. In this regard, cytokines may have a central role, since the cytokine network is activated in many disease states associated with the euthyroid sick syndrome.[7-10] Tumor necrosis factor alpha (TNF) and interleukin-1 (IL-1) are proinflammatory mediators within this cytokine network, and both induce the release of IL-6.[10-12] TNF, IL-1, and IL-6 have been shown to modulate thyroid hormone metabolism in vivo[13-16] and in vitro.[17-20] Administration of TNF and IL-6 to humans has provided an interesting model to evaluate the induction of the euthyroid sick syndrome, which occurred within hours.[15,16] Conversely, inflammatory stimuli induced a less severe euthyroid sick syndrome in IL-6 knockout mice.[21] Considering these short-term characteristics of the changes in thyroid hormone metabolism related to TNF and IL-6, we sought to determine whether the recovery from the cytokine-induced euthyroid sick syndrome would also follow certain characteristics.

Isolated limb perfusion (ILP) with recombinant TNF (rTNF) and melphalan is an effective treatment for patients with inoperable melanoma or sarcoma.[22-24] After this procedure, when the limb is reconnected to the systemic circulation, resorption of residual TNF from the perfused limb results in high systemic TNF levels,[25] providing a model to examine the acute-phase response including the euthyroid sick syndrome. The aim of this study was to evaluate the effects of TNF on thyroid hormone metabolism with respect to the induction of and recovery from the euthyroid sick syndrome. Therefore, we measured plasma thyroid hormone concentrations in relation to TNF, IL-6, and cortisol concentrations during 7 consecutive days in six cancer patients treated with ILP with rTNF and melphalan.

From the Department of Internal Medicine I, University Hospital, Rotterdam; Department of Chemical Pathology, Erasmus University, Rotterdam; Department of Rheumatology and Department of Internal Medicine, Zuiderziekenhuis, Rotterdam; Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam; Department of Endocrinology and Metabolism, Academic Medical Center, Amsterdam; Department of Surgical Oncology, University Hospital Rotterdam–Dr. Daniel den Hoed Cancer Center, Rotterdam; and Department of Internal Medicine, St. Joseph Hospital, Veldhoven, The Netherlands.

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Address reprint requests to R.A. Feelders, MD, University Hospital, Department of Internal Medicine I, Dr. Molewaterplein 40, 3015 G.D., Rotterdam, The Netherlands.

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SUBJECTS AND METHODS

Patients

We studied three male patients with irresectable soft-tissue sarcoma and three female patients with melanoma with multiple in-transit melanoma metastases. The median age was 54 years (range, 45 to 83). The study protocol was approved by the Medical Ethics Committee of Dr. Daniel den Hoed Cancer Center. Informed consent was obtained from all patients.

ILP

The method for ILP has been described previously in detail.[22-24] Briefly, the surgical procedure is performed under general anesthesia using intravenous administration of propofol (Zeneca, Caponago, Italy), pancuronium bromide (Organon Technica, Oss, The Netherlands), and sufentanil (Janssen Pharmaceutica, Beerse, Belgium) and inhalation of isoflurane (Abbott, Queensborough, England), nitrous oxide, and oxygen. The extracorporeal circuit is primed with erythrocyte concentrate and fresh frozen plasma diluted with Hartman electrolyte solution and polygeline solution (Haemaccel; Hoechst, Mannheim, Germany). Heparin (Novo-Nordisk, Rud, Norway) was used as an anticoagulant both in the priming solution (3 IU/mL) and systemically (200 IU/kg body weight). After cannulation of the local vasculature, perfusion is performed via the extracorporeal circuit at mild hyperthermia (40°C). The limb is perfused for 90 minutes with rTNF (Boehringer, Ingelheim, Germany; 2 mg per arm or 4 mg per leg) and melphalan (Wellcome, Beckenham, England; 13 mg/L arm vol and 10 mg/L leg vol), which is administered after 30 minutes. ILP is followed by drainage of the perfusate from the limb and a washout procedure with 2 to 4 L 6% dextran 70 solution (Macrodex; NBPI, Amstelveen, The Netherlands). Subsequently, the limb is reconnected to the systemic circulation and 1 mg/kg protamine sulfate (Novo-Nordisk, Rud, Norway) is administered to antagonize heparin. Perioperatively, all patients receive 2 U erythrocyte concentrate to replace blood loss due to the washout procedure in the limb and 3 L isotonic saline intravenously. After the ILP procedure, patients are extubated and observed at the intensive care unit for 24 hours. Postoperatively, prophylactic anticoagulation with acenocumarol (Ciba-Geigy, Basel, Switzerland) is started, as well as subcutaneous administration of 5,000 U calparin (Sanofi Winthrop, Nôtredame de Bondeville, France) twice daily until adequate anticoagulation is achieved.

Plasma Sampling

Blood was obtained from a peripheral vessel that was not located in the perfused limb or used for fluid administration. Baseline samples were collected 24 hours before ILP. At the completion of the ILP, defined as the moment of reconnection of the limb to the systemic circulation by releasing the tourniquet, plasma was collected at 5, 10, and 30 minutes and 4 hours and subsequently on each of 7 consecutive days at 8 AM.

Laboratory Procedures

All measurements were performed in duplicate, and all samples from each individual were measured in the same analysis.

Cytokines. TNF levels were measured using a specific sandwich enzyme-linked immunosorbent assay (ELISA) as described previously.[26] Normal TNF values were less than 50 pg/mL. IL-6 levels were measured by a specific sandwich ELISA as described previously,[27] with a detection limit of 10 pg/mL.

Hormones. Plasma T4 (reference value, 70 to 150 nmol/L; intraassay variation, 3.0%; interassay variation, 5.1%), T3 (1.3 to 2.7 nmol/L; intraassay variation, 4.0%; interassay variation, 6.3%), and rT3 (0.11 to 0.44 nmol/L; intraassay variation, 4.6%; interassay variation, 4.6%) levels were measured with in-house radioimmunoassays.[28] FT4 was assayed using the SPAC FT4-fraction (Byk-Sangtec Diagnostica, Dietzenbach, Germany; 10.0 to 23.0 pmol/L; intraassay variation, 2.8%; interassay variation, 5.7%). T3-resin uptake was determined by an MMA kit (Kodak Clinical Diagnostics, Amersham, England; 0.84 to 1.11; intraassay variation, 2.7%; interassay variation, 2.3%). The TSH level was measured by a time-resolved fluoroimmunoassay (Delfia hTSH Ultra; Wallac Oy, Turku, Finland; 0.4 to 4.0 mU/L; interassay variation, 3% to 6%). Thyroxine-binding globulin (TBG) concentrations were determined by a commercial radioimmunoassay (Eiken Chemical, Tokyo, Japan; 200 to 650 nmol/L; interassay variation, 8 to 10%). Cortisol levels were measured by a fluorescence polarization immunoassay on a TDx analyzer (Abbott Laboratories, North Chicago, IL; 0.22 to 0.65 μmol/L; interassay variation, 5% to 9%).

Statistical Analysis

Data are presented as the mean ± SEM. The effects of TNF administration on the time course of various parameters were tested by one-way ANOVA for repeated measures or Friedman's repeated-measures ANOVA on ranks when appropriate. For each variable, the data were compared at time points after TNF treatment versus baseline using Dunnett's test for multiple comparisons. A P value less than .05 was considered statistically significant.

RESULTS

Clinical Features

All patients developed chills and fever after ILP, which was treated with 1 g paracetamol rectally. The mean body temperature increased from 37.0° ± 0.1°C before ILP to 39.0° ± 0.3°C at 4 hours postperfusion, and decreased subsequently to 37.2° ± 0.1°C 1 day after ILP. At baseline, the mean arterial pressure (MAP) was 98 ± 5 mm Hg, and during approximately 2 to 4 hours after ILP, a slight decrease in MAP was observed to a minimum value of 90 ± 3 mm Hg. There was no need for treatment with vasopressors.

Cytokines and Cortisol

The changes with time for plasma TNF, IL-6, and cortisol concentrations are shown in Fig 1. Baseline levels of TNF and IL-6 before ILP were less than the detection limit in all patients. At the end of ILP (the moment of reconnection of the limb to the systemic circulation), TNF appeared in the circulation and increased to a maximum value at 10 minutes (19,980 ± 4,669 pg/mL). Subsequently, TNF levels rapidly decreased, and at 24 hours TNF was not detectable in any patients. IL-6 started to increase at 5 minutes after ILP and reached a maximal concentration at 4 hours (2,713 ± 715 pg/mL, $P < .05$). Thereafter, IL-6 decreased, and at day 2 IL-6 values were not different from pretreatment values. Baseline cortisol concentrations were within the normal range. There was a parallel time course for cortisol and IL-6: a rapid increase at 5 minutes (0.86 ± 0.15 μmol/L) followed by a peak level at 4 hours postperfusion (1.41 ± 0.23 v 0.52 ± 0.08 μmol/L at baseline, $P < .05$). Cortisol levels were not different from pretreatment values from day 1 to day 7.

Thyroid Hormones, TBG, and TSH

Pretreatment levels of thyroid hormones, TBG, and TSH were all within the normal range for our laboratory and are

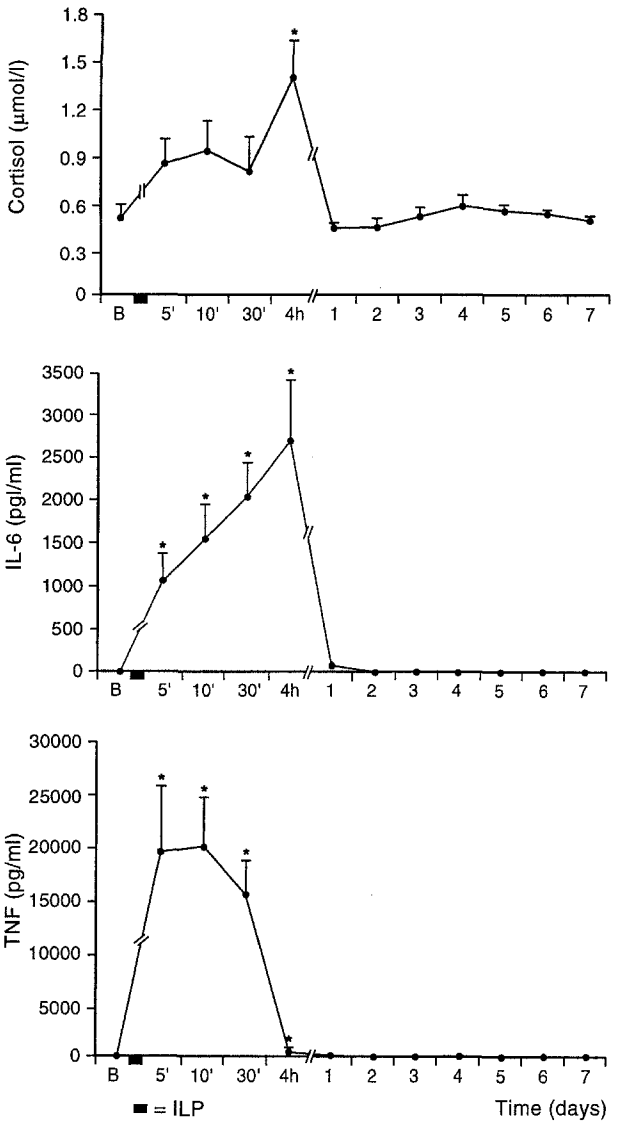

Fig 1. Time course of plasma TNF, IL-6, and cortisol concentrations after ILP with rTNF and melphalan. Measurements were performed at baseline (B) and after the end of ILP at 5, 10, and 30 minutes (') and 4 hours (h). Subsequently, samples were obtained on 7 consecutive days after ILP. Data are the mean ± SEM. *$P < .05$ v B.

shown in Table 1. There were no differences in the response of thyroid hormones between patients with sarcoma and those with melanoma. The time course for thyroid hormones, TBG, and TSH is presented in Fig 2. ILP was followed by a rapid decrease in TBG within 5 minutes postperfusion (188 ± 20 *v* 337 ± 38 nmol/L at baseline, $P < .05$). TBG concentrations remained low until day 1 and were not different from pretreatment values from day 2 to day 7. T4 levels paralleled TBG concentrations, reflected in a decrease at 5 minutes (71 ± 10 *v* 103 ± 12 nmol/L at baseline, $P < .05$), low values at day 1, and subsequent recovery at day 2. Moreover, from day 5 to day 7, an increase of T4 higher than the basal level was observed ($P < .05$).

In contrast to total T4, FT4 showed a marked increase from 16.0 ± 1.2 at baseline to 56.9 ± 9.6 pmol/L at 5 minutes postperfusion ($P < .05$). After 10 minutes, FT4 levels gradually decreased and were not different from pretreatment values from day 1 to day 7. Similarly, the pattern of T3-resin uptake was characterized by a transient increase to maximum levels at 5 minutes ($P < .05$). From day 1 to day 7, values for T3-resin uptake were not different from pretreatment values.

After ILP, a decrease was observed for both T3 (from 1.77 ± 0.13 to 0.70 ± 0.07 nmol/L, $P < .05$) and rT3 (from 0.33 ± 0.05 to 0.10 ± 0.03 nmol/L, $P < .05$) at 5 minutes postperfusion. Subsequently, T3 concentrations remained low compared with pretreatment values until day 1, whereas rT3 increased over pretreatment values to a maximum concentration at day 1 (0.56 ± 0.12 nmol/L, $P < .05$). Subsequently, T3 and rT3 levels recovered gradually, both returning to initial values at day 4.

The time course of TSH showed an initial decrease after ILP, with minimum levels at 4 hours (from 1.10 ± 0.19 to 0.72 ± 0.19 mU/L, $P < .05$). Subsequently, a marked increase over pretreatment levels was observed at day 1 (1.99 ± 0.46 mU/L, $P < .05$), preceding the recovery of T4 and T3 concentrations in all subjects. TSH remained elevated compared with pretreatment levels from day 1 to day 7 postperfusion (relative increase from +94% ± 43% to +155% ± 66%, $P < .05$).

DISCUSSION

We studied the induction of and subsequent recovery from nonthyroidal illness induced by ILP with high-dose rTNF and melphalan in cancer patients. ILP was accompanied by a period of systemic illness characterized by fever and a slight decrease in blood pressure. rTNF leakage into the systemic circulation was associated with the induction of mediators like IL-6 and cortisol and with major changes in plasma thyroid hormone concentrations compatible with the euthyroid sick syndrome.

There are only a few data available on the recovery from the euthyroid sick syndrome.[6] We show that the initial phase of recovery from the acute euthyroid sick syndrome following TNF infusion occurs rapidly, ie, within 1 day, reflected in increased TSH values. In all patients, the recovery of T3 and T4

Table 1. Plasma Concentrations of Thyroid Hormones, TBG, and TSH at Baseline in Six Cancer Patients Before ILP With rTNF and Melphalan

Subject No.	TBG (nmol/L)	T4 (nmol/L)	FT4 (pmol/L)	T3-Resin Uptake	T3 (nmol/L)	rT3 (nmol/L)	TSH (mU/L)
1	340	80	11.9	0.99	1.95	0.22	1.30
2	390	135	17.9	1.04	2.00	0.30	1.60
3	410	135	14.9	0.96	2.20	0.24	1.60
4	430	115	16.7	1.01	1.55	0.55	0.71
5	240	75	13.9	1.11	1.45	0.44	0.89
6	210	75	20.5	1.34	1.45	0.24	0.52
Mean ± SEM	337 ± 38	103 ± 12	16.0 ± 1.2	1.08 ± 0.06	1.77 ± 0.13	0.33 ± 0.05	1.10 ± 0.19
Reference value	200-650	70-150	10-23	0.8-1.1	1.3-2.7	0.11-0.44	0.4-4

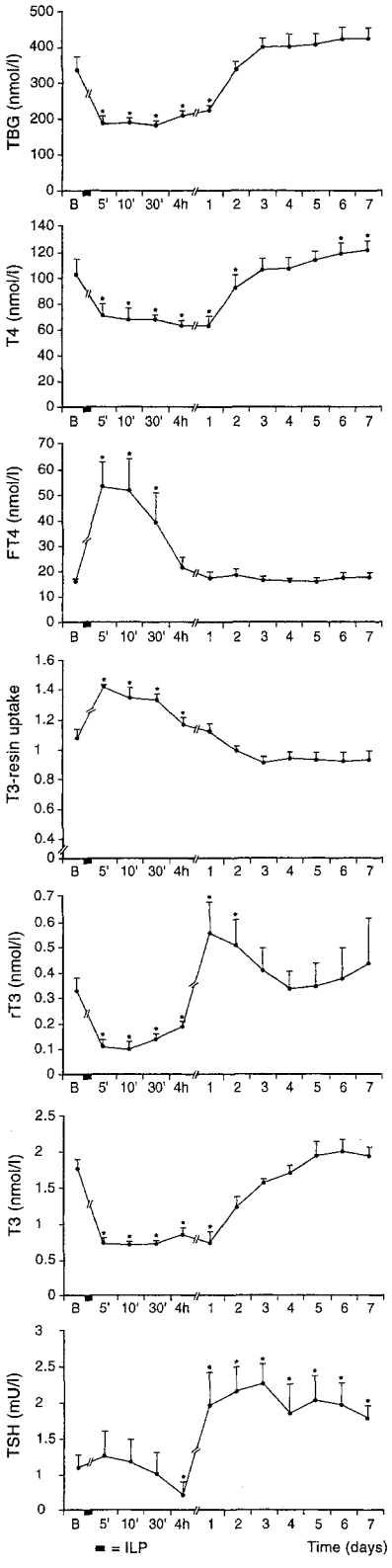

Fig 2. Time course of plasma TSH, T3, rT3, T3-resin uptake, FT4, T4, and TBG concentrations after ILP with rTNF and melphalan. Measurements were performed at baseline (B) and after the end of ILP at 5, 10, and 30 minutes (') and 4 hours (h). Subsequently, samples were obtained on 7 consecutive days after ILP. Data are the mean ± SEM. *$P < .05$ *v* B.

to pretreatment levels was preceded by a persistent and considerable increase in TSH. This temporal relationship may imply that an increase in TSH is a prerequisite for the recovery of thyroid hormone concentrations in nonthyroidal illness. This is in agreement with the study by Hamblin et al,[6] who demonstrated a concordance between a transient TSH increase and the return of T4 to normal levels in critically ill patients. In contrast to their study, we observed that the recovery phase is associated with an increase in T4 higher than the pretreatment values, which may be facilitated by the persisting elevation of TSH. This elevation of TSH and T4 concentrations occurred in the presence of FT4 and T3 values that were within the range of pre-TNF values. Therefore, feedback relationships within the hypothalamus-pituitary-thyroid axis are altered during recovery from the euthyroid sick syndrome compared with the induction phase of the syndrome and with pretreatment relationships.

The regulation of TSH secretion is altered in the euthyroid sick syndrome.[1,2] TSH values are usually normal in nonthyroidal illness and seem inappropriately low in the face of reduced T3 and T4 levels. The initial decrease of TSH levels in our study may be explained by the preceding increase of both FT4 and cortisol concentrations, known to suppress TSH secretion.[1] In addition, TNF and/or IL-6 may have decreased TSH levels.[15,16] For instance, in rats, TNF induces a decrease in both hypothalamic TRH content and pituitary TSH expression.[29] Thus, TNF and IL-6 may alter pituitary-thyroid relationships in the euthyroid sick syndrome, resulting in a blunted TSH responsiveness.

Several mechanisms may be involved in the induction of changes in thyroid hormones in our patients. We documented high systemic TNF levels after ILP, due to resorption of residual rTNF from the affected limb into the circulation.[25] It could be argued that endogenous TNF production triggered by the surgical procedure itself contributed to circulating TNF levels. However, in early studies with ILP without rTNF, systemic TNF levels were only minimally elevated, ie, about 0.1% of the TNF levels measured after infusion of TNF.[30] In addition, it has been shown previously that minor limb surgery under general anesthesia is not accompanied by detectable TNF or IL-6 levels.[31] TNF stimulates the synthesis of other cytokines like IL-6 in vivo and in vitro.[10-12] This is illustrated by the present data showing a rapid release of IL-6 after the appearance of TNF in the circulation. Concurrently, plasma cortisol levels increased, paralleling TNF and IL-6 levels. This cortisol response may be mediated by activation of the hypothalamic-pituitary-adrenal axis at multiple levels by TNF, IL-6, and possibly other inflammatory mediators.[32-35] Increased cortisol levels also may have affected thyroid hormone metabolism, eg, by suppressive effects on TSH secretion and inhibition of T4-T3 conversion.[1] FT4 concentrations showed a dramatic increase directly after ILP, which may be explained, at least in part, by the effects of heparin. Heparin administration increases FT4 levels through an in vitro artifact caused by lipoprotein lipase–mediated generation of free fatty acids (FFAs).[2,36] Alternatively, it may be speculated that mediators like TNF, IL-6, and cortisol affect T4 binding to TBG, eg, by induction of FFA release in vivo. For instance, Van der Poll et al[15] found a transient increase of FT4, albeit within the normal range, and FFA after TNF administration to healthy subjects who did not receive heparin.

We cannot exclude the possibility that the use of melphalan or anesthetics influenced our results. Unfortunately, there are no data available on the effects of these agents on thyroid hormone metabolism. The three men with sarcoma and three women with melanoma included in our study all received the same treatment. It is unlikely that this difference in sex and tumor affected our conclusions, since the changes in thyroid hormone levels were not different between the two groups.

Following ILP, TBG levels promptly decreased, associated with an acute decrease of T4, T3, and rT3 concentrations. Previously, we described the effects of ILP with rTNF on acute-phase proteins.[25] Directly after ILP, most plasma protein concentrations decrease to approximately 60% of initial values, presumably due to capillary leakage and hemodilution after fluid administration. Positive acute-phase proteins like α1-antitrypsin and α1-acid glycoprotein recover within 4 hours after ILP, followed by a subsequent increase. In contrast, the levels of negative acute-phase proteins such as albumin and transferrin remain low until day 2, which may result from inhibition of protein synthesis.[25] In this respect, TBG seems to behave like a negative acute-phase protein. For instance, IL-6, a major regulator of acute-phase protein gene expression,[37,38] inhibits TBG synthesis by a human hepatoma cell line in vitro.[39] Nonetheless, the initial decrease in TBG is likely caused by capillary leakage and hemodilution, although we cannot exclude the possibility that inhibition of TBG synthesis, eg, by IL-6, contributed to the decrease in TBG levels.

Plasma T4 levels decreased acutely after TNF infusion, predominantly related to the decrease in T4-binding proteins like TBG. Whether impaired thyroidal T4 release is also involved remains speculative. In vitro, TNF and IL-6 have inhibitory effects on TSH-stimulated thyroid cell functions like ^{125}I incorporation, thyroglobulin and thyroid peroxidase synthesis, and thyroid hormone release.[17-20] Moreover, TSH levels decreased, which may have contributed to the prolonged decrease of T4 concentrations via reduced stimulation of thyroid function.

TNF infusion was followed by induction of the euthyroid sick syndrome, as reflected in low T4 and T3 levels and elevated rT3 levels at 24 hours postperfusion. It could be argued that cancer itself can induce an euthyroid sick syndrome. However, baseline values for thyroid hormones and TSH before TNF administration were within the normal range, which may be explained by the fact that our patients had local tumors without systemic involvement. Kinetic studies in patients with the euthyroid sick syndrome have shown that low T3 levels are primarily caused by decreased peripheral conversion of T4 to T3, since thyroidal T3 production and T3 clearance are unaffected.[40,41] Conversely, rT3 production is normal and elevated rT3 levels are caused by a decreased clearance.[42,43] Several mechanisms may underlie these reciprocal changes in T3 and rT3 levels, with a possible role for cytokines. First, this involves an impaired hepatic type I 5′-deiodinase (5′-DI) activity.[44] In rats, TNF inhibits hepatic 5′-DI activity after 1 day of treatment.[29] However, the effects of cytokines on human 5′-DI expression are presently unknown. Second, hepatic T4 and rT3 uptake may be inhibited in nonthyroidal illness by factors such as FFA and bilirubin.[45-47] Cytokines may also modulate hepatic thyroid hormone processing; however, this needs further investigation.

In conclusion, our results show that the euthyroid sick syndrome is part of the acute-phase response to ILP with rTNF and melphalan. The recovery from this acute euthyroid sick syndrome starts early and is associated with an elevation of TSH and T4, but not T3 and FT4. These data illustrate that the recovery from nonthyroidal illness, such as that induced by TNF, has typical features with respect to thyroid hormone metabolism. The euthyroid sick syndrome should be interpreted not only in relation to the presence of nonthyroidal diseases but also in relation to recovery from these diseases.

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