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Reduced Activation and Increased Inactivation of Thyroid Hormone in Tissues of Critically Ill Patients

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Critical illness is often associated with reduced TSH and thyroid hormone secretion as well as marked changes in peripheral thyroid hormone metabolism, resulting in low serum T_3 and high rT_3 levels. To study the mechanism(s) of the latter changes, we determined serum thyroid hormone levels and the expression of the type 1, 2, and 3 iodothyronine deiodinases (D1, D2, and D3) in liver and skeletal muscle from deceased intensive care patients. To study mechanisms underlying these changes, 65 blood samples, 65 liver, and 66 skeletal muscle biopsies were obtained within minutes after death from 80 intensive care unit patients randomized for intensive or conventional insulin treatment. Serum thyroid parameters and the expression of tissue D1-D3 were determined. Serum TSH, T_4, T_3, and the T_3/rT_3 ratio were lower, whereas serum rT_3 was higher than in normal subjects ($P < 0.0001$). Liver D1 activity was down-regulated and D3 activity was induced in liver and skeletal muscle. Serum T_3/rT_3 ratio correlated positively with liver D1 activity ($P < 0.001$) and negatively with liver D3 activity (ns). These parameters were independent of the type of insulin treatment. Liver D1 and serum T_3/rT_3 were highest in patients who died from severe brain damage, intermediate in those who died from sepsis or excessive inflammation, and lowest in patients who died from cardiovascular collapse ($P < 0.01$). Liver D3 showed an opposite relationship. Acute renal failure requiring dialysis and need of inotropes were associated with low liver D1 activity ($P < 0.01$ and $P = 0.06$) and high liver D3 ($P < 0.01$) and skeletal muscle D3 ($P < 0.05$) activity. Liver D1 activity was negatively correlated with plasma urea ($P = 0.002$), creatinine ($P = 0.06$), and bilirubin ($P < 0.0001$). D1 and D3 mRNA levels corresponded with enzyme activities (both $P < 0.001$), suggesting regulation of the expression of both deiodinases at the pretranslational level. This is the first study relating tissue deiodinase activities with serum thyroid hormone levels and clinical parameters in a large group of critically ill patients. Liver D1 is down-regulated and D3 (which is not present in liver and skeletal muscle of healthy individuals) is induced, particularly in disease states associated with poor tissue perfusion. These observed changes, in correlation with a low T_3/rT_3 ratio, may represent tissue-specific ways to reduce thyroid hormone bioactivity during cellular hypoxia and contribute to the low T_3 syndrome of severe illness. (*J Clin Endocrinol Metab* 88: 3202–3211, 2003)

CRITICAL ILLNESS INDUCES a variety of hormonal changes that differ between acute and prolonged critical illness (1). Striking alterations observed in acute critical illness include increased serum catecholamines, GH, and cortisol levels, a blunted GH pulsatility, insulin resistance, and the so-called low T3 syndrome (1, 2). In prolonged critical illness, catecholamine and cortisol levels decrease, compared with the acute situation, and a decrease in the levels of GH, TSH, and thyroid hormone occurs. There are no indications that the acute changes are harmful, but it is unclear whether the endocrine changes in prolonged critical illness are all beneficial adaptations because recent data suggest that some may contribute to a worsening of the clinical condition (3).

Substitution of critically ill patients with high doses of GH, corticosteroids, or thyroid hormone has been found to have no or even a negative effect on clinical outcome (4–7). In a recent study, it was shown that strict control of blood glucose levels less than 110 mg/dl with intensive insulin therapy markedly reduces morbidity and mortality in critically ill patients (8). Endocrine intervention with hypothalamic releasing factors, which restores pulsatile pituitary hormone secretion and normalizes peripheral hormone levels, may be another successful approach (3, 9).

In this study we focused on the peripheral metabolism of thyroid hormone in critically ill patients. In critical illness, serum T_3 concentration decreases and serum rT_3 increases, the magnitudes of these changes being related to the severity of disease (10). Although serum T_4 and free T_4 (FT4) may be increased in mild illnesses, serum T_4 is decreased, and FT4 normal or decreased in severely ill patients (10).

In humans, peripheral thyroid hormone metabolism is mediated importantly by the three iodothyronine deiodinases D1, D2, and D3 (see Refs. 11 and 12 for reviews). D1 is present in liver, kidney, and thyroid and plays a key role in the production of serum T_3 from T_4 and in the breakdown of the metabolite rT_3 (11, 12). D2 is present in brain, pituitary, thyroid, and skeletal muscle and also converts T_4 by outer-ring deiodination to T_3. In tissues such as the brain, D2 is important for local T_3 production, but the enzyme in skeletal muscle may also contribute to plasma T_3 production (11, 12). D3 is present in brain, skin, placenta, pregnant uterus, and

Abbreviations: 3,3′-T2, 3,3′-diiodothyronine; Ct, cycle threshold; D1, D2, and D3, type 1, 2, and 3 iodothyronine deiodinase; DTT, dithiothreitol; FT4, free T_4; ICU, intensive care unit; MOF, multiple organ failure; PED, phosphate, EDTA; PTU, 6-n-propyl-2-thiouracil; RRT, renal replacement therapy; RT, reverse transcription; SIRS, systemic inflammatory response syndrome.

various fetal tissues; it catalyzes the inactivation of T_4 and T_3 by inner-ring deiodination to rT3 and 3,3′-diiodothyronine (3,3′-T2), respectively (11, 12).

The strongly reduced circulating T_3 levels in sick patients may be due in part to a decreased peripheral T_4 deiodination by D1, D2, or both (12–14). The increase in serum rT_3 levels is explained by a decrease in D1 activity because D1 is the principal pathway for rT_3 clearance (15). Besides a decreased D1 activity, an impaired transport of T_4 and rT_3 into D1-containing tissues such as liver may be another important mechanism for the changes in thyroid hormone levels associated with illness (16). However, the possibility that an increased D3 activity contributes to the reduced serum T_3 levels and increased rT_3 levels should also be considered. Patients with D3-expressing hemangiomas may have very low serum T_4 and T_3 concentrations, combined with very high serum rT_3 levels (17). The term consumptive hypothyroidism is used for this syndrome. There are hitherto no data on the eventual role of D3 induction in the low T_3 syndrome of severe illness.

In this study, serum samples and liver and skeletal muscle biopsies were obtained from 80 patients within minutes after they died in a surgical intensive care unit (ICU). The patients had been randomized for intensive or conventional insulin therapy as recently described (8). Tissue D1, D2, and D3 gene expression and activity as well as serum TSH, T_4, T_3, and rT_3 concentrations were measured. The correlations among these analytes as well as their correlations with several clinical parameters were calculated.

Materials and Methods

Materials

Nonradioactive iodothyronines were obtained from Henning (Berlin, Germany). [3′,5′-^{125}I]T4 (with a specific activity of ca. 2000 mCi/μmol) was obtained from Amersham Pharmacia (Rozendaal, The Netherlands). [3′-^{125}I]T3 and [3′,5′-^{125}I]rT3 (with both a specific activity of ca. 2000 mCi/μmol) were prepared by radioiodination of 3,5-T2 and 3,3′-T2, respectively (18). [^{125}I]T4 and [^{125}I]rT3 were purified immediately before use by Sephadex LH-20 (Amersham Pharmacia) chromatography (19). N-bromoacetyl-[^{125}I]T3 (BrAc[^{125}I]T3) was prepared as previously described (20). Its purity was checked by HPLC analysis. Protein molecular weight markers, and 6-n-propyl-2-thiouracil (PTU) were obtained from Sigma (Zwijndrecht, The Netherlands); dithiothreitol (DTT) from ICN (Zoetermeer, The Netherlands); electrophoresis grade SDS-PAGE reagents from Bio-Rad (Veenendaal, The Netherlands); Coomassie Brilliant Blue R-250 from Merck (Darmstadt, Germany); and TaqMan probes and primers from Biosource (Nivelles, Belgium). All other chemicals used in this study were of reagent grade.

Subjects

This study was part of a large randomized, controlled study on intensive insulin treatment in ICU patients (n = 1548), of which the major clinical outcomes have been published in detail elsewhere (8). On admission, patients were randomly assigned to either strict normalization of blood glucose (80–110 mg/dl) with intensive insulin therapy or the conventional approach, in which insulin infusion is initiated only when blood glucose exceeds 215 mg/dl, to maintain blood glucose levels between 180 and 200 mg/dl. Maximal insulin dose was arbitrarily set at 50 IU/h. The study protocol has been approved by the Ethical Review Board of the University of Leuven School of Medicine, and patients were included after informed consent from the closest family member.

A total of 80 patients was included in this study. Blood samples were obtained from 65 patients, liver biopsies from 66 patients, and skeletal muscle (rectus abdominis) biopsies from 66 patients within minutes after death [25.2 ± 20.0 (SD), range (5–97 min) for liver and 20.7 ± 19.7, range (0–95 min) for skeletal muscle]. From 51 patients blood, liver, and skeletal muscle samples were available. All patients had been randomized for conventional or intensive insulin treatment (8). Thirty-one patients had been treated with thyroid hormone during the course of their critical illness when they had a serum T_4 concentration less than 50 nmol/liter in the face of a normal thyroxine-binding globulin and concomitantly clinical symptoms of hypothyroidism, defined as coma or central nervous system suppression, failure to wean from the ventilator, or hemodynamic instability, which were unexplained and resistant to conventional supportive therapy. In these cases, thyroid hormone treatment consisted of an iv bolus of 150 μg T_4 daily plus 0.6 μg T_3 per kg body weight per 24 h as a continuous iv infusion. All patients included in this study had died in the ICU, and the cause of death was determined both clinically by the attending ICU physician and postmortem examination. The pathologist was unaware of insulin treatment allocation. Relevant patients' characteristics are summarized in Table 1.

Serum analyses

The care of patients in the ICU often comprises infusion of heparin, either systemically or locally to prevent clotting of vascular access, which substantially interferes with the assay used to quantify free concentrations of thyroid hormone (21). Therefore, we refrained from measuring serum FT4 and free T_3 in this study. Serum total T_4, total T_3, and TSH were measured by chemoluminescence assays (Vitros ECi Immunodiagnostic System, Ortho-Clinical Diagnostics, Amersham, UK). The rT3 was measured by RIA as previously described (22). Within-assay coefficients of variation amounted to 4% for TSH, 2% for T_4, 2% for T_3, and 3–4% for rT_3. Normal values for TSH, T_4, T_3, and rT_3 were determined in 270 healthy individuals. Mean ± 2 SD was used as the normal range for T_4, T_3, and rT_3, whereas the 95% confidence interval was used for TSH.

TABLE 1. Patients' characteristics, divided in different groups based on cause of death

	Cause of death[a]				
	I	II	III	IV	Total
No. of patients	11	36	27	6	80
Age (yr)	66.6 ± 11.1	70.2 ± 12.4	68.7 ± 12.8	62.1 ± 9.0	68.6 ± 12.1
BMI	28.4 ± 6.1	24.9 ± 3.5	25.2 ± 5.4	23.6 ± 3.0	25.4 ± 4.7
ICU stay (days)	8.7 ± 7.3	31.4 ± 34.5	24.9 ± 25.4	6.2 ± 5.7	24.2 ± 28.9
Male	5	26	20	4	55
Intensive insulin therapy	6	6	11	2	25
Thyroid hormone treatment	4	15	12	0	31
RRT[b]	4	20	14	2	40
Inotropes treatment	10	32	21	3	66

Data represent mean ± SD.

[a] I, Cardiovascular collapse; II, MOF sepsis; III, MOF SIRS; IV, severe brain damage.

[b] RRT, renal replacement therapy: hemodialysis or hemofiltration.

Deiodinase activities

Human liver and skeletal muscle samples were homogenized on ice in 10 volumes of PE buffer [0.1 M phosphate and 2 mM EDTA (pH 7.2)] using a Polytron (Kinematica AG, Lucerne, Switzerland). Homogenates were snap frozen in aliquots and stored at −80 C until further analysis. Protein concentration was measured with the Bio-Rad protein assay using BSA as the standard following the manufacturer's instructions.

Liver D1 activities were determined as described earlier (23) by duplicate incubations of homogenates (10 μg protein) for 30 min at 37 C with 0.1 μM [3′,5′-^{125}I]rT3 (100,000 cpm) in a final volume of 0.1 ml PED10 buffer (PE + 10 mM DTT). To validate the specificity of the D1 assay, some incubations were also carried out in the presence of 0.1 mM of the D1 inhibitor PTU or excess unlabeled rT_3 (1 μM). Reactions were stopped by addition of 0.1 ml 5% (wt/vol) BSA in water on ice. The protein-bound iodothyronines were precipitated by addition of 0.5 ml ice-cold 10% (wt/vol) trichloroacetic acid in water. Following centrifugation, $^{125}I^-$ was isolated from the supernatant by chromatography on Sephadex LH-20 minicolumns (24). Skeletal muscle D1 activities were assayed similarly, using 200 μg homogenate protein in a 60-min incubation.

Liver and skeletal muscle D2 activities were assayed as earlier described (25) by duplicate incubation of 200 μg homogenate protein for 60 min at 37 C with 1 nM [3′,5′-^{125}I]T4 (100,000 cpm) in a final volume of 0.1 ml PED25 buffer (PE + 25 mM DTT). The incubations were carried out in the presence of 0.1 μM unlabeled T_3, to prevent inner-ring deiodination of the labeled T_4 substrate by D3, if present, and in the absence or presence of 0.1 μM unlabeled T_4, which is sufficient to saturate D2. Deiodination of labeled T4 in the absence minus that in the presence of excess unlabeled T_4 represents D2 activity. The further procedure for the quantitation of $^{125}I^-$ production was the same as described above for the D1 assay.

Tissue D3 activities were measured as described earlier (24) by duplicate incubation of liver (100 μg protein) or skeletal muscle (200 μg protein) homogenate for 60 min at 37 C with 1 nM [3′-^{125}I]T_3 (200,000 cpm) in a final volume of 0.1 ml PED50 buffer. To validate the D3 assay, some incubations were also carried out in the presence of 10 or 100 nM unlabeled T_3. Reactions were stopped by addition of 0.1 ml ice-cold methanol. After centrifugation, 0.15 ml of the supernatant was added to 0.1 ml 0.02 M ammonium acetate (pH 4), and 0.1 ml of the mixture was applied to a 4.6 × 250 mm Symmetry C18 column connected to an Alliance HPLC system (Waters, Etten-Leur, The Netherlands). The column was eluted with a linear gradient of acetonitrile (28%–42% in 15 min) in 0.02 M ammonium acetate (pH 4.0) at a flow of 1.2 ml/min. The radioactivity in the eluate was measured on-line using a Radiomatic A-500 flow scintillation detector (Packard, Meriden, CT).

Affinity labeling

Affinity labeling of liver D1 was done as previously described (23) in samples with low, intermediate, or high D1 activity. Briefly, homogenate (100 μg protein) was incubated for 15 min at 37 C with 100,000 cpm (~25 fmol) BrAc[^{125}I]T3 in 75 μl PED1 buffer (PE + 1 mM DTT). To specifically block affinity labeling of D1 protein, some incubations were carried out in the presence of 10 μM rT_3 and 100 μM PTU (23). Labeling was stopped by the addition of SDS-loading buffer and heating the mixture for 5 min at 80 C. Proteins (100 μg per lane) were separated by SDS-PAGE gel electrophoresis in a 12% resolving, 5% stacking gel (23). Gels were stained with Coomassie Brilliant Blue R-250, dried at 80 C under vacuum, and autoradiographed by exposure overnight at −80 C to Biomax imaging film (Eastman Kodak, Rochester, NY).

RNA isolation and reverse transcription (RT)

RNA was isolated from liver samples using the High Pure RNA tissue kit (Roche Diagnostics, Almere, The Netherlands) according to the manufacturer's protocol. RNA concentrations were determined using the RiboGreen RNA quantitation kit (Molecular Probes, Leiden, The Netherlands). All samples were diluted to 0.1 μg/μl, and 1 μg was used for cDNA synthesis using the TaqMan RT kit (Roche Diagnostics).

Real-time RT-PCR

D1 and D3 mRNA levels were determined in a set of liver samples with low, intermediate, and high deiodinase activities. The ABI PRISM 7700 sequence detection system (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands) was used, which uses TaqMan chemistry for highly accurate quantitation of mRNA levels. Sequences and concentrations of the primers and probes are given in Table 2. Hepatic D1 and D3 mRNA levels are expressed relative to those of the glyceraldehyde-3-phosphate dehydrogenase housekeeping gene. The glyceraldehyde-3-phosphate dehydrogenase probe and primers were provided as preoptimized control system (Applied Biosystems).

Reactions were done for 2 min at 50 C and for 10 min at 95 C, followed by 40 cycles of 15 sec at 95 C and for 1 min at 60 C. According to the manufacturer's guidelines, data were expressed as cycle threshold (Ct) values, which represent the cycle number at which probe-derived dye absorbance reaches the calculated threshold value. Data are expressed as ΔCt (*i.e.* the Ct value of the target gene minus the Ct value of the housekeeping gene).

Statistical analysis

Data were analyzed using the statistical program SPSS 10.0.7 for Windows (SPSS Inc., Chicago, IL). Logarithmic transformations were applied to normalize variables and minimize the influence of outliers, when appropriate. All analyses were done on the whole group as well as on subgroups treated or not treated with thyroid hormone. Data were analyzed using one-way ANOVA tests, with a *post hoc* Fisher's least significant difference test for multiple comparisons, *t* tests, Mann-Whitney *U* tests, and linear regression analyses, when appropriate.

Results

Compared with the normal ranges for our laboratory, serum TSH, T_4, and T_3 levels and the T_3/rT_3 ratio were low, whereas mean serum rT_3 was high ($P < 0.0001$) (Table 3). Patients who were treated with thyroid hormone had higher serum T_3 and lower TSH levels than patients who were not treated with thyroid hormone. Serum T_4 and rT_3 levels were not different between these groups. No significant correlation was observed among serum TSH, T_4, T_3, and rT_3 and postmortem time.

Significant D1 activities were measured in all liver samples, with a range of 0.44–17.5 pmol/min per milligram protein. In the liver samples tested, deiodination of the substrate rT_3 was completely blocked by addition of the D1-specific inhibitor PTU and was largely saturated by increasing the rT_3 concentration from 0.1 to 1.1 μM. The approximate

TABLE 2. Sequences and concentrations of primers and probes that were used for determination of D1 and D3 mRNA levels by quantitative real-time RT-PCR

Primers and probes	Sequence	Concentration
D1 forward	5′-TTAGTTCCATAGCAGATTTTCTTGTCA-3′	200 nM
D1 reverse	5′-CTGATGTCCATGTTGTTCTTAAAAGC-3′	200 nM
D1 probe	5′-FAM-AGCCATCTGATGCATGTGCTTCTTCAATG-TAMRA-3′	100 nM
D3 forward	5′-TTCCAGAGCCAGCACATCCT-3′	200 nM
D3 reverse	5′-ACGTCGCGCTGGTACTTAGTG-3′	200 nM
D3 probe	5′-FAM-TGCACCTGACCACCGTTCATGGC-TAMRA-3′	200 nM

TABLE 3. Descriptive statistics of thyroid hormone levels and deiodinase activities in this population, subdivided in a group not receiving thyroid hormone (TH) treatment and a group receiving TH treatment

	TH treatment	Mean	Median	SD	Range	Normal values	P^a
TSH (mU/liter)		0.75	0.17	1.58	(0.001–10.7)	0.2–4.2	<0.0001
	No (n = 39)	1.12	0.61	1.91	(0.003–10.7)		
	Yes (n = 25)	0.18[b]	0.01	0.48	(0.001–2.15)		
T_4 (nmol/liter)		46.3	40.6	28.8	(5.4–121)	58–128	<0.0001
	No (n = 39)	45.2	38.8	30.5	(5.4–121)		
	Yes (n = 25)	48.0	44.7	26.4	(9.6–98.1)		
T_3 (nmol/liter)		1.24	0.98	0.79	(0.41–4.71)	1.43–2.51	<0.0001
	No (n = 39)	1.01	0.78	0.76	(0.41–4.71)		
	Yes (n = 25)	1.60[b]	1.41	0.72	(0.67–3.21)		
rT3 (nmol/liter)		1.85	1.37	2.15	(0.22–15.78)	0.14–0.34	<0.0001
	No (n = 39)	1.75	1.13	2.56	(0.22–15.78)		
	Yes (n = 25)	1.99	1.85	1.31	(0.41–5.44)		
T_3/rT3 (molar ratios)		1.23	0.83	1.20	(0.18–6.13)	4.2–17.9	<0.0001
	No (n = 39)	1.25	0.70	1.28	(0.18–6.13)		
	Yes (n = 25)	1.20	0.90	1.07	(0.31–4.81)		
Liver D1 (pmol/mg · min)		4.51	3.25	3.89	(0.44–17.53)		
	No (n = 44)	4.57	3.33	3.65	(0.52–16.78)		
	Yes (n = 21)	4.40	2.55	4.43	(0.44–17.53)		
Liver D3 (fmol/mg · min)		1.04	0.60	1.43	(0.14–9.15)		
	No (n = 44)	0.97	0.51	1.19	(0.15–5.60)		
	Yes (n = 21)	1.18	0.71	1.87	(0.14–9.15)		
Muscle D3 (fmol/mg · min)		0.23	0.14	0.29	(0.06–1.65)		
	No (n = 43)	0.26	0.13	0.32	(0.06–1.65)		
	Yes (n = 22)	0.23	0.16	0.24	(0.09–1.14)		

rT3, Reverse T_3. To convert values for T_4 to micrograms per deciliter, divide by 12.87; to convert values for T_3 and rT3 to nanograms per deciliter, divide by 0.0154.

[a] P values (t test) represent serum thyroid parameters of this population compared with normal values used in our laboratory.

[b] $P < 0.01$ *vs.* untreated group.

Michaelis constant value for rT_3 was 0.4 ± 0.1 μM (mean ± SD, n = 4), which is in good agreement with previous data (11, 12). Negligible D1 activities were observed in the skeletal muscle homogenates. D2 activities were undetectable in all liver and skeletal muscle biopsies.

Significant D3 activities were detected in most tissue samples, with ranges of 0.1–9.2 and 0.1–1.7 fmol/min per milligram protein in liver and skeletal muscle, respectively. In both tissues, D3 activity was progressively saturated by increasing the T_3 concentration from 1 to 10 and 100 nM, providing approximate Michaelis constant values of 3.6 ± 0.6 nM T_3 in liver and 2.3 ± 0.6 nM T_3 for skeletal muscle (mean ± SD, n = 4). These values are in close agreement with previous reports (11, 12). There was a significant correlation between D3 expression in liver and skeletal muscle (R = 0.54, $P < 0.001$), but in some patients high expression in liver was observed with low expression in skeletal muscle and vice versa. No significant correlation was observed between deiodinase activities and postmortem time.

Serum thyroid hormone levels and tissue deiodinase activities were not different between the patients who had received intensive or conventional insulin therapy (Table 4). This has been found in the whole group and separately in the groups of patients who did or did not receive thyroid hormone treatment.

All regression analyses were also performed on the whole group and separately on the groups of patients who did or did not receive thyroid hormone treatment. Unless mentioned specifically, similar correlations were observed in the treated and untreated groups. The serum T_3/rT_3 ratio showed a positive correlation with liver D1 activity (linear regression test, R = 0.66, $P < 0.001$) and a negative, insignificant correlation with liver D3 activity ($P = 0.17$) (Fig. 1) Serum T_3/rT_3 was not correlated with muscle D3 activity.

The possible relationships of tissue deiodinase activities with serum iodothyronine parameters other than the T_3/rT_3 ratio were also analyzed. Surprisingly, liver D1 activity showed a positive correlation with serum T_4. This was due to the strong relationship in thyroid hormone-treated patients (R = 0.74, $P = 0.002$), whereas no correlation was seen in patients who had not received thyroid hormone ($P = 0.49$). Liver D1 activity showed a negative correlation with serum rT_3 (R = −0.48, $P < 0.001$) and the serum rT_3/T_4 ratio (R = −0.55, $P < 0.001$), independent of thyroid hormone treatment. Liver D1 activity was not correlated with serum T_3 but showed an unexpected negative correlation with the serum T_3/T_4 ratio that was stronger in patients who had been treated *vs.* those who had not been treated with thyroid hormone (R = −0.68, $P = 0.005$ *vs.* R = −0.44, $P = 0.008$).

Liver D3 activity showed a positive correlation with serum rT_3 (R = 0.40, $P = 0.016$) and the rT_3/T_4 ratio (R = 0.55, $P = 0.001$) in the group of patients who had not been treated with thyroid hormone. These correlations were completely absent in the group of patients who had received thyroid hormone treatment. Skeletal muscle D3 activity showed a positive correlation with the serum rT_3/T_4 ratio (R = 0.55, $P = 0.001$). No other correlations were found for liver or skeletal muscle D3 activity with serum iodothyronine levels.

Hepatic D1 activity showed a significant correlation with cause of death, being lowest in the patients who had died of a cardiovascular collapse, with successive increases in the patients who had died of multiple organ failure (MOF) with sepsis or MOF with systemic inflammatory response syndrome (SIRS) and being highest in the patients who had died

TABLE 4. The effect of insulin treatment on serum thyroid parameters and deiodinase activities

	Intensive insulin treatment	Mean	Median	SD	Range	Normal values	Mann-Whitney *U P*[a]
TSH (mU/liter)						0.2–4.2	
	No (n = 48)	0.93	0.21	1.79	0.001–10.7		0.08
	Yes (n = 16)	0.23	0.05	0.30	0.002–0.79		
T_4 (nmol/liter)						58–128	
	No (n = 48)	25.3	43.1	28.0	5.4–121		0.66
	Yes (n = 16)	49.3	37.4	31.7	18.8–118		
T_3 (nmol/liter)						1.43–2.51	
	No (n = 48)	1.19	0.96	0.68	0.41–3.21		0.64
	Yes (n = 16)	1.40	1.14	1.08	0.51–4.71		
rT3 (nmol/liter)						0.14–0.34	
	No (n = 48)	1.73	1.52	1.32	0.24–5.44		0.48
	Yes (n = 16)	2.19	1.37	3.72	0.22–15.78		
T_3/rT3 (molar ratio)						4.2–17.9	
	No (n = 48)	1.21	0.72	1.28	0.18–6.13		0.34
	Yes (n = 16)	1.27	1.09	0.91	0.30–3.29		
Liver D1 (pmol/mg · min)							
	No (n = 43)	4.46	3.25	4.12	0.52–17.53		0.73
	Yes (n = 22)	4.61	3.21	3.48	0.44–11.44		
Liver D3 (fmol/mg · min)							
	No (n = 43)	1.18	0.60	1.72	0.15–9.15		0.93
	Yes (n = 22)	0.76	0.62	0.46	0.14–1.84		
Muscle D3 (fmol/mg · min)							
	No (n = 42)	0.28	0.14	0.36	0.06–1.65		0.24
	Yes (n = 23)	0.15	0.13	0.07	0.06–0.30		

rT3, Reverse T_3. To convert values for T_4 to micrograms per deciliter, divide by 12.87; to convert values for T_3 and rT3 to nanograms per deciliter, divide by 0.0154.

[a] *P* values (Mann-Whitney *U* test) represent intensive insulin treatment *vs.* conventional insulin treatment.

of severe brain damage (ANOVA $P < 0.01$). Liver D1 activities in the latter group were similar to those determined in normal liver samples (data not shown). The serum T_3/rT_3 ratio showed a similar positive correlation with cause of death (ANOVA $P < 0.01$), whereas liver D3 activity showed an insignificant negative correlation (ANOVA $P = 0.2$) with cause of death. These relationships were strongest in the group of patients who had not been treated with thyroid hormone (Fig. 2), but similar correlations were also found in the group of patients who had been treated with thyroid hormone. D3 activities in skeletal muscle did not correlate with cause of death.

Liver D1 activity was significantly lower in patients with acute renal failure requiring renal replacement therapy (RRT) such as dialysis or hemofiltration and in those receiving inotropes, compared with those who did not require these treatments (Mann-Whitney *U* test, $P < 0.01$ for both) (Figs. 3 and 4). Liver D3 activity was higher in patients who had been treated with inotropes (Mann-Whitney *U* test, $P < 0.05$) or RRT ($P = 0.056$) (Figs. 3 and 4). Muscle D3 activity was also higher in patients who had been treated with inotropes (Mann-Whitney *U* test, $P < 0.05$) (Fig. 4). Correction for treatment with dopamine did not affect these correlations. Liver D1 activity was negatively correlated with plasma urea ($R = -0.35$, $P = 0.01$) and with plasma creatinine ($R = -0.27$, $P = 0.06$) (Fig. 5). A remarkably strong, negative correlation was found for liver D1 activity with plasma total bilirubin ($R = -0.54$, $P < 0.0001$) (Fig. 6). D3 activities in liver and skeletal muscle showed no relationship with plasma urea, creatinine, or bilirubin levels. No correlation was found of liver D1, liver D3, or skeletal muscle D3 activity with plasma C-reactive protein levels.

Affinity labeling of the 27-kDa D1 protein in liver homogenates using BrAc[^{125}I]T_3 showed a good correlation with the D1 activities determined in the same samples (Fig. 7). Affinity labeling of D1 but not of other proteins was blocked by the addition of PTU and unlabeled rT_3 (not shown), in support of the specificity of the affinity labeling of D1 with BrAc[^{125}I]T_3. Liver D1 and D3 mRNA levels determined by real-time quantitative RT-PCR were significantly correlated with the corresponding deiodinase activities ($R = 0.74$, $P < 0.001$, for D1; $R = 0.78$, $P < 0.001$ for D3) (Fig. 8).

Discussion

Critical illness is associated with reduced TSH and thyroid hormone secretion as well as with marked changes in peripheral thyroid hormone metabolism, resulting in low circulating T_3 and high rT_3 levels (10). Both the fall in serum T_3 and the rise in rT_3 have been found to correlate with severity of illness (10) and decreased serum T_4 has been associated with poor prognosis. Inactivation of thyroid hormone is presumed to reflect an adaptive response of the body to conserve tissue function. As expected, we found low serum levels of TSH, total T_4 and T_3, high levels of rT_3, and a low active over inactive thyroid hormone (T_3/rT_3) ratio in the studied critically ill patients. Liver D1 activity was down-regulated and liver and skeletal muscle D3 activity, not present in healthy individuals, was induced. Changes in tissue deiodinase activities, particularly pronounced in conditions characterized by low tissue perfusion, were statistically correlated with the altered circulating thyroid hormone levels, suggesting a role in the pathophysiology of the low T3 syndrome of severe illness. Previous studies have suggested large changes in serum thyroid hormone levels after death (26). However, correlations observed in our study cannot be explained by

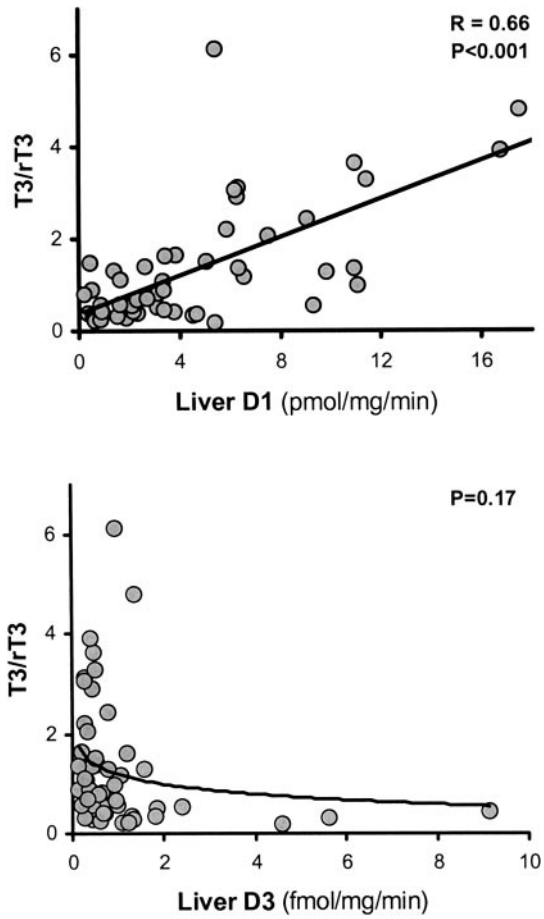

FIG. 1. Correlation of liver D1 (A) and liver D3 (B) activities with the serum T_3/rT_3 ratio in 65 patients. No distinction is made in this figure between patients who received thyroid hormone treatment and patients who did not. Liver D1 shows a significant positive correlation with T_3/rT_3 ratio ($P < 0.001$), whereas liver D3 shows an insignificant negative trend ($P = 0.17$).

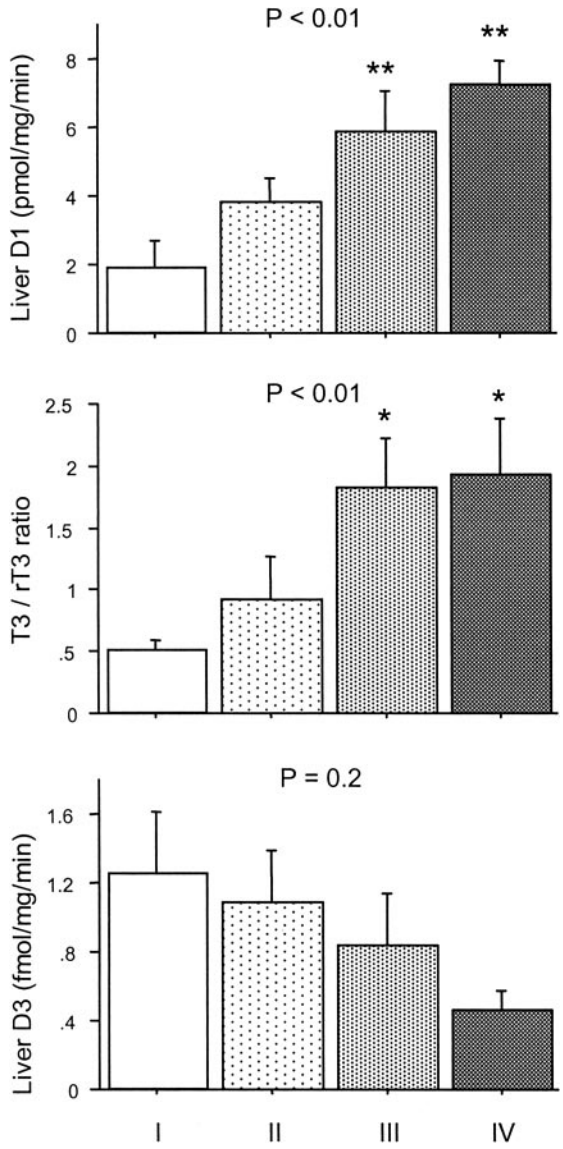

FIG. 2. Correlation of liver D1 (A) and liver D3 (B) activities, and the T_3/rT_3 ratio (C) with cause of death in patients who were not treated with thyroid hormone. Patients are divided into four different groups based on cause of death. I, Cardiovascular collapse (n = 5); II, multiple organ failure with sepsis (n = 21); III, multiple organ failure with systemic inflammatory response syndrome (n = 14); IV, severe brain damage (n = 4). Liver D1 activity and serum T_3/rT_3 ratio showed a significant relation with cause of death ($P < 0.01$), whereas liver D3 activity showed an opposite trend. **, $P < 0.01$ *vs.* group I; *, $P < 0.05$ *vs.* group I. Data represent means ± SEM and *P* values were obtained with ANOVA and Fisher's least significant difference for multiple comparisons.

differences in postmortem time because no relation of postmortem time with deiodinase activities or serum thyroid parameters was observed.

In healthy subjects, about 20% of circulating T_3 is secreted by the thyroid. Estimates of the contribution of liver and kidney D1 to peripheral T_3 production vary from 15% to 80% (12), with the remainder of extrathyroidal T_3 production originating in D2-containing tissues such as skeletal muscle. The contribution of D1 seems to be highest in hyperthyroid patients, whereas D2 may play a more important role in euthyroid and, in particular, hypothyroid subjects (12). A low D1 activity will result in not only a decreased production of T_3 from T_4 but also reduced clearance of rT_3 because D1 is the principal pathway for rT_3 clearance (12). A decreased uptake of T_4 and rT3 into D1-expressing tissues is another possible mechanism to lower serum T_3 and increase serum rT_3 levels (10, 15, 16). Furthermore, the activity of the different deiodinases greatly depends on thiol cofactors, although the physiological cofactor(s) for each deiodinase has not been identified (11, 12). In our study, tissue deiodinase activities were determined in the presence of excess exogenous cofactor (DTT). Our studies, therefore, do not provide information about changes in the availability of the natural cofactors as a possible mechanism for the changes in peripheral thyroid hormone metabolism in critical illness.

In normal healthy subjects, only D1 is expressed in liver and preliminary data suggest that D2 activity is expressed in skeletal muscle (12). In liver of critically ill patients, we found D1 activity, which, except for patients who died acutely from severe brain damage, was low, compared with values observed in healthy individuals, and also substantial D3 activity. As expected, D2 activity could not be detected in liver.

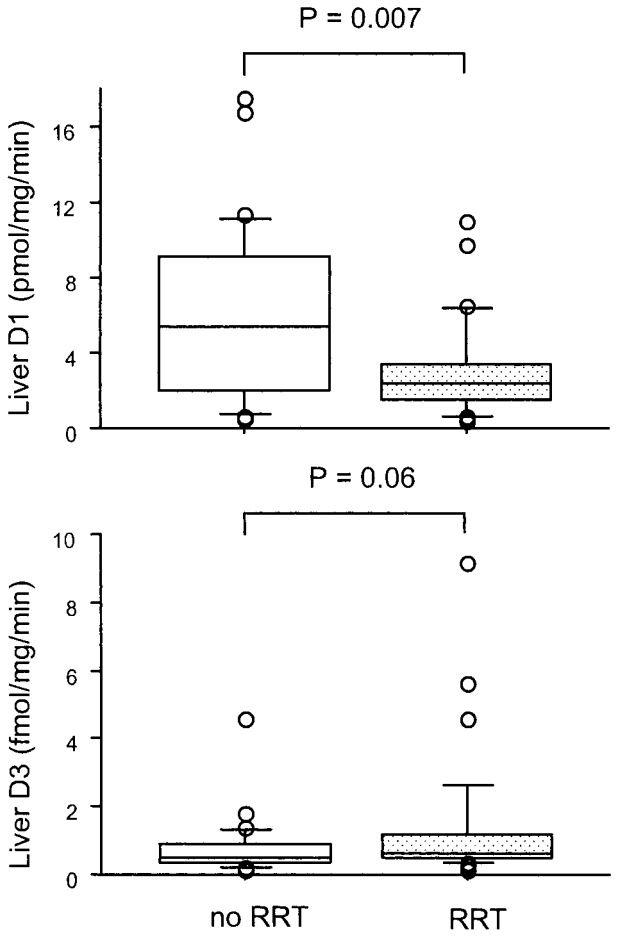

FIG. 3. Liver D1 (A) and liver D3 (B) activities in patients with acute renal failure requiring RRT (n = 33) and patients who did not require this treatment (n = 32). Liver D1 was significantly lower in patients who required RRT ($P < 0.01$), whereas liver D3 activity was higher ($P = 0.06$). Box plots represent 10th-25th-50th-75th-90th percentile, and P values were obtained with Mann-Whitney U test.

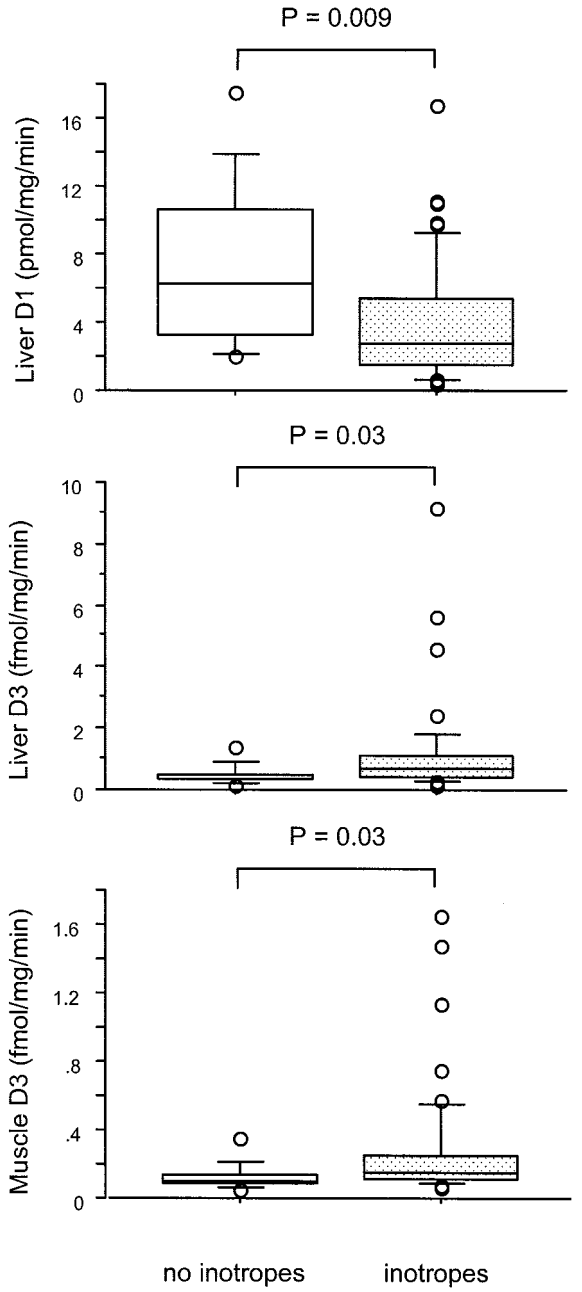

FIG. 4. Liver D1 (A) and liver D3 (B) activities in patients receiving inotropes (n = 54) and patients who did not require this treatment (n = 11). Liver D1 was significantly lower in patients who required inotropes ($P < 0.01$), whereas liver D3 activity was higher ($P < 0.01$). Skeletal muscle D3 activity (C) was also higher ($P < 0.05$) in patients who were treated with inotropes (n = 53) than in patients who were not (n = 12). Box plots represent 10th-25th-50th-75th-90th percentiles, and P values were obtained with Mann-Whitney U test.

Surprisingly, skeletal muscle of critically ill patients showed a significant D3 activity, whereas both muscle D1 and D2 activities were negligible. In general, the expression of D1 and D3 is up-regulated and that of D2 is down-regulated by thyroid hormone (11, 12). Based on the low serum thyroid hormone levels in our patients, a high D2 expression and a low D1 and D3 expression would be expected. Although we did observe mostly low hepatic D1 expression, D2 activity in skeletal muscle was undetectable, and liver and skeletal muscle samples expressed substantial D3 activities. Hence, a role of regulators other than thyroid hormone is suggested.

To reduce the confounding effect of variable concentrations of T_4 and T_4-binding proteins, we mainly focused on the correlation of tissue deiodinase activities with serum iodothyronine ratios. Because a low D1 expression conceivably reduces T_3 production and rT_3 clearance and because elevated D3 expression enhances T_3 clearance and rT_3 production, the serum T_3/rT_3 ratio is the parameter that most accurately reflects the result of altered peripheral thyroid hormone metabolism during critical illness. Our data suggest an important role of liver D1 in inducing the altered thyroid hormone levels of critically ill patients because liver D1 activity was positively correlated with serum T_3/rT_3 ratio and negatively correlated with the serum rT_3/T_4 ratio. Surprisingly, liver D1 activity also showed a negative correlation with the serum T_3/T_4 ratio. This is due in part to the positive correlation between liver D1 activity and serum T_4 levels in the thyroid hormone-treated group, which may be explained by the positive control of D1 expression by thyroid hormone, although this is thought to be mediated by T_3 (12, 27). Liver

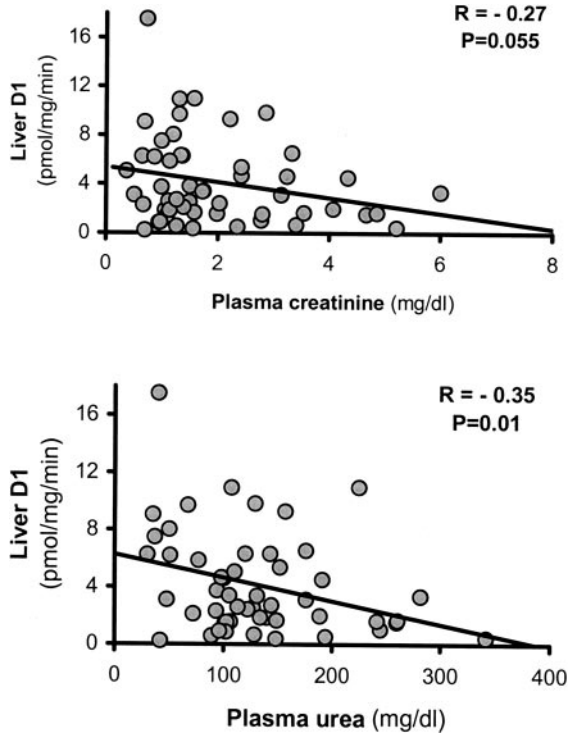

FIG. 5. Correlation of liver D1 activity with plasma creatinine and plasma urea levels (on the last day) in 65 patients. Liver D1 activity showed a negative trend with plasma creatinine ($P = 0.06$) and a negative correlation with plasma urea levels ($P = 0.01$).

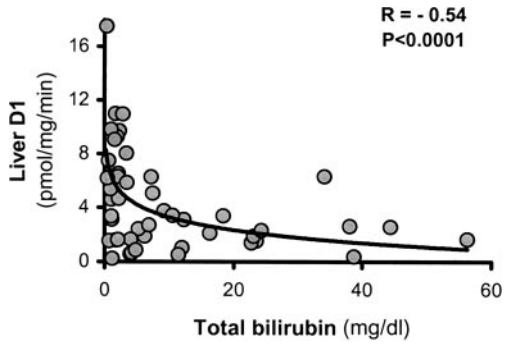

FIG. 6. Correlation of liver D1 activity with serum bilirubin levels (on the last day) in 65 patients. Liver D1 activity showed a significant negative correlation with serum bilirubin levels ($P < 0.0001$).

D3 activity correlated positively with serum rT_3/T_4 and tended to correlate negatively with serum T_3/rT_3. Normally, D3 is present only in human liver during fetal development, in which it protects the fetus from undue exposure to thyroid hormone, suggesting that pathological conditions in adult life may be associated with changes in deiodinase expression, in particular that of D3, to levels occurring during fetal development (24, 28). There are two ways by which D3 decreases T_3 availability: It prevents conversion of T_4 to T_3 by catalyzing the conversion of T_4 to rT_3 instead, and it also catalyzes the degradation of T_3 to 3,3′-T2. Therefore, induction of liver D3 in critically ill patients is likely to contribute to the low serum T_3 and high serum rT_3 levels in critically ill patients.

There is evidence that D2 is expressed in skeletal muscle of healthy subjects (25). D2 activity is regulated by substrate-induced enzyme inactivation (12). Hence, it may be surprising that we failed to detect significant D2 activity in skeletal muscle of critically ill patients, particularly in view of the low serum T_4 levels. However, critically ill patients have strongly increased serum rT_3 concentrations, which may also inactivate D2 (29). Another explanation for the lack of D2 activity is the short half-life of functional D2 protein (<1 h in euthyroid conditions), which may cause rapid postmortem D2 inactivation (12). However, because in this study tissue biopsies were taken within minutes after death, postmortem decay of D2 activity should be minimal. The shortest interval between entry in the ICU and isolation of tissue samples was between 24 and 48 h. Thus, in view of the short half-life of the D2 protein, it may well have disappeared from skeletal muscle if its expression is acutely suppressed in severe illness. It is also possible that D2 expression varies between different types of skeletal muscle, but this remains to be investigated. Like D1, D2 is a thyroid hormone-activating enzyme. Therefore, the lack of D2 activity in skeletal muscle, as does the down-regulation of hepatic D1 activity, may contribute to low serum T_3 levels in critically ill patients.

Another surprising finding was the expression of substantial D3 activity in skeletal muscle. Muscle D3 activity was significantly correlated with serum rT_3/T_4 ratio, but not with T_3/rT_3 ratio, in critically ill patients. Although D3 activity has been reported previously in fetal rat muscle (30), this is the first report of D3 expression in human skeletal muscle. By converting T_4 to rT_3 and T_3 to 3,3′-T2, D3 may lower local thyroid hormone levels in skeletal muscle. It has been shown recently that expression of D3 in hemangiomas may result in very low serum T_4 and T_3 and very high rT_3 levels (17). Because skeletal muscle is such an abundant tissue in humans, it is likely that induction of D3 in skeletal muscle also contributes to the low serum T_4 and T_3 and high rT3 levels in critical illness.

No significant differences in tissue deiodinase expression were observed between patients who had been treated with intensive or conventional insulin therapy. This seems to contradict the marked beneficial effects of intensive insulin therapy on morbidity and mortality in intensive care patients (8). However, it should be noted that the current study involved postmortem samples. The illness of patients who died after being intensively treated with insulin was therefore at least as severe as that of patients who died after having received conventional insulin therapy.

Liver D1 activity and serum T_3/rT_3 ratio showed a significant relationship with cause of death. Cause of death was categorized in four different groups, with group 1 having the most severe, and group 4 the least hemodynamic instability before death. Hepatic D1 activity and serum T_3/rT_3 were lowest in patients who died of a cardiovascular collapse (group 1) and were highest in patients who died of severe brain damage (group 4). Liver D3 showed an opposite, insignificant correlation with cause of death. Because ICU stay was shorter in groups 1 and 4 than in groups 2 and 3 (MOF with sepsis and MOF with SIRS), these correlations cannot be explained by the duration of the illness. These data suggest that the observed changes in liver deiodinase activity may

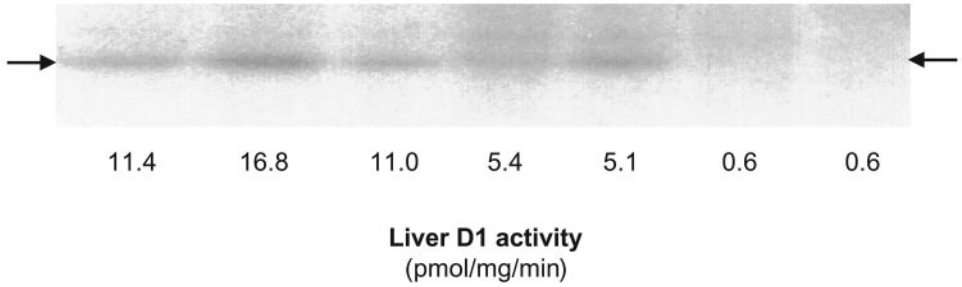

FIG. 7. Affinity labeling of the 27-kDa protein in liver homogenates using BrAc[^{125}I]T3. Liver D1 activity is shown on the bottom of the figure and shows a good correlation with the affinity labeling of the 27-kDa D1 protein *(arrow)*.

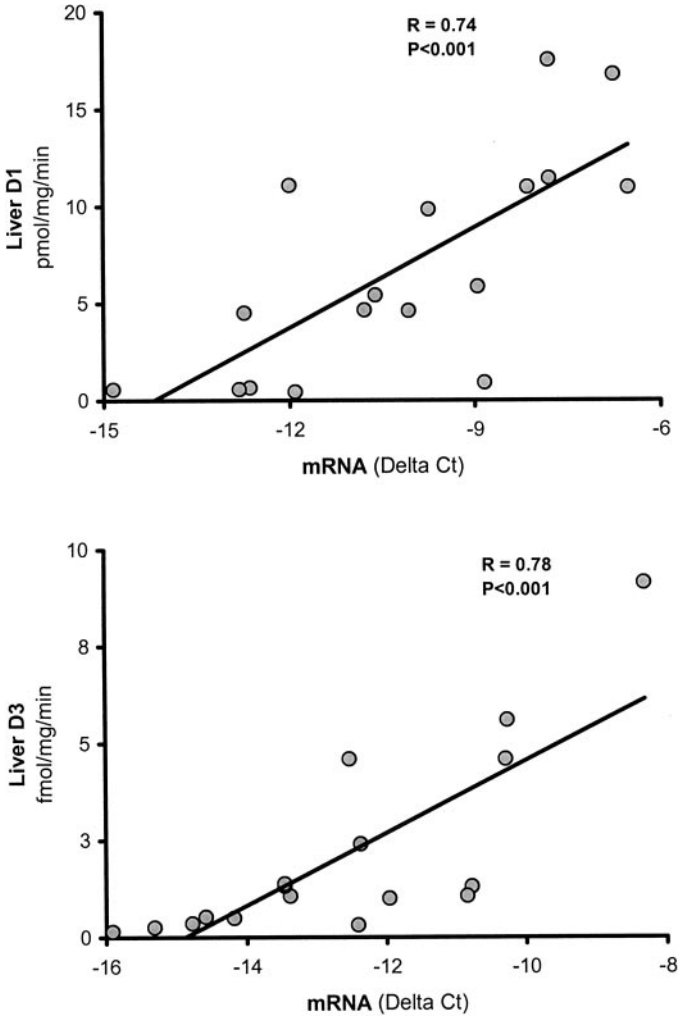

FIG. 8. Correlation of liver D1 (A) and liver D3 (B) activities with D1 and D3 mRNA levels in a selection of samples with low, intermediate, or high activity. The mRNA levels were determined by real-time RT-PCR and are expressed as Δ Ct, *i.e.* the Ct value of the target gene minus the Ct value of the housekeeping gene. Liver D1 and D3 activities showed positive correlations with corresponding mRNA levels ($P < 0.001$).

relate to poor tissue perfusion. For this reason, we analyzed the relation of tissue deiodinase activities with several other clinical parameters related to a decreased tissue perfusion, such as decreased kidney function (plasma urea and creatinine), the need for renal replacement therapy (dialysis or hemofiltration), the need for inotropes, and impaired liver function (plasma total bilirubin). Liver D1 activity was negatively correlated with plasma urea and creatinine. Patients with acute renal failure requiring dialysis/hemofiltration and those requiring inotropes for hemodynamic stability had lower liver D1 activities and higher liver D3 activities than patients who did not require these treatments. Skeletal muscle D3 activity was also higher in patients treated with inotropes. Although dopamine is known to inhibit TSH secretion and peripheral T_4 to T_3 conversion (31), correction for dopamine did not affect these correlations. Liver D1 activity showed a very strong negative correlation with plasma total bilirubin, whereas no correlation was shown with inflammation as reflected by an elevated CRP level. To our knowledge, there are no previous data suggesting a relation between deiodinase activity and tissue hypoxia, but a pH dependence of D1 activity has been shown in perfused rat livers (32). Because D1 is responsible for the activation and D3 for the inactivation of thyroid hormone, regulation of deiodinase activities by cellular hypoxia may be a tissue-specific way to alter thyroid hormone bioactivity during limited oxygen supply.

Our data regarding hepatic deiodinase activities were substantiated by a good correlation with liver D1 and D3 mRNA levels and affinity labeling of the D1 protein with N-bromoacetyl-[^{125}I]T3. This suggests that the changes in liver D1 and D3 expression in severely ill patients are largely exerted at the pretranslational level.

In conclusion, this is the first report on the relationships among tissue deiodinase activities, thyroid hormone levels, and clinical parameters in a large group of critically ill patients. Liver D1 activity is down-regulated, and liver and skeletal muscle D3, both not present in healthy subjects, are induced, particularly in those disease states with poor tissue perfusion and unrelated to inflammation. The data suggest that low D1 activity plays an important role in the altered thyroid hormone levels during critical illness. The induction of D3 in liver and skeletal muscle of critically ill patients suggests a role of this enzyme in pathophysiology, which may be more important than previously thought. Indeed, high D3 activity in an abundant tissue such as skeletal muscle is likely to lower circulating T4 and T3 and increase rT3 levels, hence contributing to the low T3 syndrome of severe illness.

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References

1. **Van den Berghe G** 2002 Dynamic neuroendocrine responses to critical illness. Front Neuroendocrinol 23:370–391
2. **Ligtenberg JJ, Girbes AR, Beentjes JA, Tulleken JE, van der Werf TS, Zijlstra JG** 2001 Hormones in the critically ill patient: to intervene or not to intervene? Intensive Care Med 27:1567–1577
3. **Van den Berghe G, Baxter RC, Weekers F, Wouters P, Bowers CY, Iranmanesh A, Veldhuis JD, Bouillon R** 2002 The combined administration of GH-releasing peptide-2 (GHRP-2), TRH and GnRH to men with prolonged critical illness evokes superior endocrine and metabolic effects compared to treatment with GHRP-2 alone. Clin Endocrinol (Oxf) 56:655–669
4. **Takala J, Ruokonen E, Webster NR, Nielsen MS, Zandstra DF, Vundelinckx G, Hinds CJ** 1999 Increased mortality associated with growth hormone treatment in critically ill adults. N Engl J Med 341:785–792
5. **Bone RC, Fisher Jr CJ, Clemmer TP, Slotman GJ, Metz CA, Balk RA** 1987 A controlled clinical trial of high-dose methylprednisolone in the treatment of severe sepsis and septic shock. N Engl J Med 317:653–658
6. **Slotman GJ, Fisher Jr CJ, Bone RC, Clemmer TP, Metz CA** 1993 Detrimental effects of high-dose methylprednisolone sodium succinate on serum concentrations of hepatic and renal function indicators in severe sepsis and septic shock. The Methylprednisolone Severe Sepsis Study Group. Crit Care Med 21:191–195
7. **Brent GA, Hershman JM** 1986 Thyroxine therapy in patients with severe nonthyroidal illnesses and low serum thyroxine concentration. J Clin Endocrinol Metab 63:1–8
8. **Van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P, Bouillon R** 2001 Intensive insulin therapy in critically ill patients. N Engl J Med 345:1359–1367
9. **Van den Berghe G** 1999 Growth hormone secretagogues in critical illness. Horm Res 51(S3):21–28
10. **Docter R, Krenning EP, de Jong M, Hennemann G** 1993 The sick euthyroid syndrome: changes in thyroid hormone serum parameters and hormone metabolism. Clin Endocrinol (Oxf) 39:499–518
11. **Leonard JL, Koehrle J** 2000 Intracellular pathways of iodothyronine metabolism. In: Braverman LE, Utiger RD, eds. Werner and Ingbar's the thyroid: a fundamental and clinical text. Philadelphia: Lippincott, Williams &. Wilkins; 136–173, ed 8
12. **Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR** 2002 Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr Rev 23:38–89
13. **Kaptein EM, Robinson WJ, Grieb DA, Nicoloff JT** 1982 Peripheral serum thyroxine, triiodothyronine and reverse triiodothyronine kinetics in the low thyroxine state of acute nonthyroidal illnesses. A noncompartmental analysis. J Clin Invest 69:526–535
14. **Faber J, Thomsen HF, Lumholtz IB, Kirkegaard C, Siersbaek-Nielsen K, Friis T** 1981 Kinetic studies of thyroxine, 3, 5, 3′-triiodothyronine, 3, 3, 5′-triiodothyronine, 3′, 5′-diiodothyronine, 3, 3′-diiodothyronine, and 3′-monoiodothyronine in patients with liver cirrhosis. J Clin Endocrinol Metab 53:978–984
15. **Kaptein EM, Feinstein EI, Nicoloff JT, Massry SG** 1983 Serum reverse triiodothyronine and thyroxine kinetics in patients with chronic renal failure. J Clin Endocrinol Metab 57:181–189
16. **Hennemann G, Docter R, Friesema EC, de Jong M, Krenning EP, Visser TJ** 2001 Plasma membrane transport of thyroid hormones and its role in thyroid hormone metabolism and bioavailability. Endocr Rev 224:451–476
17. **Huang SA, Tu HM, Harney JW, Venihaki M, Butte AJ, Kozakewich HP, Fishman SJ, Larsen PR** 2000 Severe hypothyroidism caused by type 3 iodothyronine deiodinase in infantile hemangiomas. N Engl J Med 343:185–189
18. **Toyoda N, Kaptein E, Berry MJ, Harney JW, Larsen PR, Visser TJ** 1997 Structure-activity relationships for thyroid hormone deiodination by mammalian type I iodothyronine deiodinases. Endocrinology 138:213–219
19. **Mol JA, Visser TJ** 1985 Synthesis and some properties of sulfate esters and sulfamates of iodothyronines. Endocrinology 117:1–7
20. **Mol JA, Docter R, Kaptein E, Jansen G, Hennemann G, Visser TJ** 1984 Inactivation and affinity-labeling of rat liver iodothyronine deiodinase with N-bromoacetyl-3, 3′, 5-triiodothyronine. Biochem Biophys Res Commun 124: 475–483
21. **Mendel CM, Frost PH, Kunitake ST, Cavalieri RR** 1987 Mechanism of the heparin-induced increase in the concentration of free thyroxine in plasma. J Clin Endocrinol Metab 65:1259–1264
22. **Visser TJ, Docter R, Hennemann G** 1977 Radioimmunoassay of reverse triiodothyronine. J Endocrinol 73:395–396
23. **Schoenmakers CH, Pigmans IG, Visser TJ** 1992 Species differences in liver type I iodothyronine deiodinase. Biochim Biophys Acta 1121:160–166
24. **Richard K, Hume R, Kaptein E, Sanders JP, van Toor H, De Herder WW, den Hollander JC, Krenning EP, Visser TJ** 1998 Ontogeny of iodothyronine deiodinases in human liver. J Clin Endocrinol Metab 83:2868–2874
25. **Salvatore D, Bartha T, Harney JW, Larsen PR** 1996 Molecular biological and biochemical characterization of the human type 2 selenodeiodinase. Endocrinology 137:3308–3315
26. **Wortsman J, Premachandra BN, Chopra IJ, Murphy JE** 1987 Hypothyroxinemia in cardiac arrest. Arch Intern Med 147:245–248
27. **Berry MJ, Kates AL, Larsen PR** 1990 Thyroid hormone regulates type I deiodinase messenger RNA in rat liver. Mol Endocrinol 45:743–748
28. **Kester MH, Kaptein E, Van Dijk CH, Roest TJ, Tibboel D, Coughtrie MW, Visser TJ** 2002 Characterization of iodothyronine sulfatase activities in human and rat liver and placenta. Endocrinology 143:814–819
29. **Steinsapir J, Bianco AC, Buettner C, Harney J, Larsen PR** 2000 Substrate-induced down-regulation of human type 2 deiodinase (hD2) is mediated through proteasomal degradation and requires interaction with the enzyme's active center. Endocrinology 1413:1127–1135
30. **Huang TS, Chopra IJ, Boado R, Soloman DH, Chua Teco GN** 1988 Thyroxine inner ring monodeiodinating activity in fetal tissues of the rat. Pediatr Res 23:196–199
31. **Van den Berghe G, de Zegher F** 1996 Anterior pituitary function during critical illness and dopamine treatment. Crit Care Med 24:1580–1590
32. **Kohrle J, Muller MJ, Kodding R, Seitz HJ, Hesch RD** 1982 pH-dependency of iodothyronine metabolism in isolated perfused rat liver. Biochem J 202: 667–675