Type 2 Iodothyronine Deiodinase in Skeletal Muscle: Effects of Hypothyroidism and Fasting


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Context: The iodothyronine deiodinases D1, D2, and D3 enable tissue-specific adaptation of thyroid hormone levels in response to various conditions, such as hypothyroidism or fasting. The possible expression of D2 mRNA in skeletal muscle is intriguing because this enzyme could play a role in systemic as well as local T3 production.

Objective: We determined D2 activity and D2 mRNA expression in human skeletal muscle biopsies under control conditions and during hypothyroidism, fasting, and hyperinsulinemia.

Design: This was a prospective study.

Setting: The study was conducted at a university hospital.

Patients: We studied 11 thyroidectomized patients with differentiated thyroid carcinoma (DTC) on and after 4 wk off T4 replacement and six healthy lean subjects in the fasting state and during hyperinsulinemia after both 14 and 62 h of fasting.

Mean Outcome Measures: D2 activity and D2 mRNA levels were measured in skeletal muscle samples.

Results: No differences were observed in muscle D2 mRNA levels in DTC patients on and off T4 replacement therapy. In healthy subjects, muscle D2 mRNA levels were lower after 62 h compared to 14 h of fasting. Insulin increased mRNA expression after 62 h, but not after 14 h of fasting. Skeletal muscle D2 activities were very low and not influenced by hypothyroidism and fasting.

Conclusion: Human skeletal muscle D2 mRNA expression is modulated by fasting and insulin, but not by hypothyroidism. The lack of a clear effect of D2 mRNA modulation on the observed low D2 activities questions the physiological relevance of D2 activity in human skeletal muscle. (J Clin Endocrinol Metab 94: 2144–2150, 2009)

Peripheral thyroid hormone metabolism is mainly regulated by the iodothyronine deiodinases D1, D2, and D3 (1, 2). D1 is expressed in liver, kidney, thyroid, and at a lower level in the pituitary. This enzyme converts the prohormone T4 to active T3 and is very active in the breakdown of rT3 (1, 3). Although D1 contributes significantly to peripheral T4 to T3 conversion, it is probably not the major source of extrathyroidal T3 production in humans (1, 2, 4, 5). D2 also catalyzes the production of T3.

Abbreviations: BAT, Brown adipose tissue; BMI, body mass index; Ct, cycle threshold; DTC, differentiated thyroid carcinoma; FT4, free T4; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
through outer ring deiodination of T₄ and is present in brain, pituitary, thyroid, brown adipose tissue (BAT) and, perhaps, skeletal muscle (1, 6–10). In brain, pituitary, and BAT, D2 is very important for local T₃ production. D3 inactivates T₃ and T₄ by inner ring deiodination (2) and is present in brain, skin, placenta, and fetal tissues (1). These deiodinases allow the adaptation of thyroid hormone levels of individual tissues in response to various conditions.

During hypothyroidism, the conversion of T₄ to T₃ by D2 is increased, whereas the activities of D1 and D3 are decreased (1, 11, 12). D2 mRNA was found to be expressed in skeletal muscle samples from healthy subjects (9, 13). Because skeletal mass is a major body compartment, muscle could therefore play a role in systemic and local T₃ production (9). Maia et al. (14) proposed that D2 is a major source of circulating T₃ in euthyroid subjects and even more so during hypothyroidism. In line with this assumption, we hypothesized that during hypothyroidism caused by withdrawal from T₄ substitution therapy in thyroidectomised patients treated for differentiated thyroid carcinoma (DTC), D2 activities might be up-regulated in skeletal muscle.

Short-term fasting induces a decrease in plasma T₃ that is most probably due to a decreased activity of D1 and/or D2 and/or an increased activity of D3 (1, 15, 16). Indeed, the fasting-induced decrease in serum T₃ levels has been attributed to lower peripheral conversion of T₄ to T₃ (17, 18). Because the fall in T₃ levels (50%) may be larger than can be accounted for by a drop in D1 activity and because D2 has an extremely short half-life, D2 activity may have an important role in the reduction in serum T₃ as well (1). In contrast to fasting, insulin has been shown to increase both D2 activity and mRNA expression in BAT in animal studies (19–21). Moreover, a recent study demonstrated that incubation of human myoblasts and myotubes with peroxisome proliferator-activated receptor-γ agonists resulted in increased D2 activity and also suggested a possible role for D2 in insulin signaling (22). We therefore hypothesized that conditions of fasting as well as hyperinsulinemia would affect skeletal muscle D2 expression and activity in vivo because these conditions affect insulin signaling (23).

To our knowledge, no studies in human skeletal muscle samples have been performed to investigate the effect of hypothyroidism or fasting and insulin on skeletal muscle deiodinase mRNA expression or D2 activity. To address this issue, we analyzed D2 activity and mRNA expression of D2 and D3 in skeletal muscle samples in thyroidectomized patients with DTC on and after 4 wk off T₄ replacement therapy and in healthy subjects in the fasting state and during hyperinsulinemia after both 14 and 62 h of fasting.

Subjects and Methods

Subjects with DTC

Patients were recruited from the outpatient clinic of the Department of Endocrinology of Leiden University Medical Center, which is a tertiary referral center for DTC. Patients were included who had been diagnosed with DTC and had received initial therapy consisting of near-total thyroidectomy and radioiodine ablation therapy. Additional therapies were allowed, as long as they resulted in cure. Cure was documented by the absence of measurable serum thyroglobulin during TSH stimulation as well as by a negative total-body scintigraphy with 4 mCi ¹³¹I. The patients had to be on TSH suppressive therapy, defined as TSH levels below the lower reference value for TSH (0.4 mU/liter). The adequacy of the TSH suppressive therapy was documented by yearly TSH measurements.

Patients who had diabetes mellitus or other endocrine diseases or had a body mass index (BMI) above 30 kg/m² were excluded. Patients who used any drugs known to influence thyroid hormone metabolism were also excluded. The ethics committee of Leiden University Medical Center approved the study, and written informed consent was obtained from all subjects.

Study design in DTC patients

Patients with DTC undergoing TSH-stimulated ¹³¹I scintigraphy were asked to participate in the study. Four weeks after T₄ withdrawal and 8 wk after subsequent T₄ replacement, patients were admitted to the clinical research unit at 0800 h. All subjects fasted from the preceding evening (1800 h) until the end of the study day. Length (meters), weight (kilograms), and BMI (weight/length²) were measured. Patients were studied in a semirecumbent position. A catheter was inserted in a dorsal hand vein to collect plasma samples for measurement of TSH, free T₄ (FT₄), T₃, and rT₃. Muscle biopsies were taken from the quadriceps muscle (vastus lateralis) under local anesthesia (Lidocaine 20 mg/ml; Fresenius, Kabi, Den Bosch, The Netherlands) as described earlier (23). One skeletal muscle biopsy obtained during hypothyroidism was lost. Biopsies were quickly washed in HEPES-buffered saline to remove blood, inspected for fat or fascia content, dried on gauze swabs, and subsequently stored in liquid nitrogen until analysis. Serum samples were handled immediately and stored at −20 C.

Fasting subjects

Six lean healthy men with a normal thyroid status who participated in a study on fasting-induced peripheral insulin resistance were included in this study (23). Written informed consent was obtained from all subjects after explanation of purpose, nature, and potential risks of the study. The study was approved by the Medical Ethical Committee of the Academic Medical Center of the University of Amsterdam.

Study design in fasting subjects

The experimental protocol has been briefly described earlier (23). In short, subjects were studied twice: after 14 and 62 h of fasting. Study days were separated by at least 1 wk. Subjects were fasting from 2000 h to 1000 h the next day or 3 d later. They were allowed to drink water at libitum.

After admission at the metabolic research unit, a catheter was inserted into an antecubital vein for sampling of venous blood for determination of plasma TSH, T₄, FT₄, T₃, and rT₃. A muscle biopsy (vastus lateralis of the quadriceps muscle) was performed as described above. Thereafter a continuous infusion of insulin (60 mU/m²/min) (Actrapid 100 IU/ml; Novo Nordisk Farma B.V., Alphen aan den Rijn, The Netherlands) and glucose 20% (to maintain a plasma glucose level of 5 mmol/liter) was started. Plasma glucose levels were measured every 5 min at the bedside. After 5 h of insulin infusion, muscle biopsies were repeated.

Thyroid parameters

Plasma and serum thyroid hormone levels of DTC patients and fasting volunteers were determined as described previously (in Refs. 24 and 25, respectively).

D2 activity

Skeletal muscle samples were homogenized on ice in 10 volumes of PED10 buffer [0.1 m phosphate (pH 7.2), 2 mM EDTA, and 10 mM dithiothreitol] using a polytron (Kinematica AG, Lucerne, Switzerland). Protein concentrations were measured with the Bio-Rad protein assay.
(Bio-Rad, Veenendaal, The Netherlands) using BSA as the standard according to the manufacturer’s protocol.

Skeletal muscle D2 activities were measured as previously described (9). Duplicates of 200 μg homogenate protein were incubated for 60 min at 37°C with 1 nmol [3,5,3-125I]T4 in a final volume of 0.1 ml PED10 buffer. The incubations were done in the absence or presence of 0.1 μM unlabeled T3, to prevent inner ring deiodination of the labeled T4 substrate by D3, if present, and in the absence or presence of 0.1 μM unlabeled T4, which is sufficient to saturate D2. Deiodination of labeled T4 in the absence minus that in the presence of excess labeled T4 represents D2 activity. Reaction products were analyzed by determination of the [125I]T3, generated by HPLC analysis of ethanol extracts of the reaction mixtures as previously described (26). The samples from the DTC patients were also analyzed by isolation of the released 125I- from the supernatant after addition of albumin and protein precipitation with 10% trichloroacetic acid.

To rule out interfering effects of local anesthesia on D2 activity in the human muscle samples, we analyzed the effects of increasing lidocaine concentrations on D2 activity expressed in COS1 cells transfected with a human D2 construct (D2-COS1 cells) in pcDNA3 as previously described (27). To rule out the presence of factors in skeletal muscle homogenates that could inhibit D2 activity, we measured D2 activity in D2-COS1 cell lysates with the addition of increasing volumes of serum (12.5–50 μl) of homogenate (50–200 μg of protein).

Quantitative mRNA analysis

RNA was isolated from skeletal muscle samples using High Pure RNA kit (Roche Diagnostics, Almere, The Netherlands) following the manufacturer’s instructions. RNA concentrations were determined using the RiboGreen RNA quantification kit (Molecular Probes, Leiden, The Netherlands). All samples were diluted to 0.1 μg/μl, and 1 μg was used for cDNA synthesis using TaqMan RT kit (Roche Diagnostics). D2 and D3 cDNA were analyzed on an ABO PRISM 7700 sequence detection system (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands), which uses TaqMan chemistry for highly accurate quantitation of mRNA levels. Sequences and concentrations of the primers are given in Table 1. The D2 and D3 mRNA levels are expressed relative to those of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or cyclophilin in the household gene GAPDH or cyclophilin (21).

Reactions were done for 2 min minimal at 50°C and for 10 min at 95°C, followed by 40 cycles of 15 sec at 95°C and 1 min at 60°C. Following the manufacturer’s guidelines, the cycle threshold (Ct) was determined, which represents the cycle number at which probe-derived dye absorbance reaches the calculated threshold value. Data were expressed as 2ΔCt, where ΔCt represents the Ct value of the housekeeping gene minus the Ct value of the target gene.

Statistical analysis

Statistical comparisons were performed with the Wilcoxon signed rank test. Differences were considered statistically significant at P < 0.05. The SPSS statistical software program version 12.0.2 (SPSS Inc., Chicago, IL) was used for statistical analysis. Data are presented as mean ± SE.

<table>
<thead>
<tr>
<th>TABLE 1. Primers and probes used for the determination of D2 and D3 mRNA levels by quantitative real-time RT-PCR</th>
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<tbody>
<tr>
<td><strong>Primers and probes</strong></td>
</tr>
<tr>
<td>D2 forward</td>
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<tr>
<td>D2 reverse</td>
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<tr>
<td>D2 probe</td>
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<tr>
<td>D3 forward</td>
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<tr>
<td>D3 reverse</td>
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<tr>
<td>D3 probe</td>
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TABLE 2. Thyroid hormone parameters in DTC patients

<table>
<thead>
<tr>
<th>Hypothyroidism (n = 11)</th>
<th>T4 treatment (n = 11)</th>
<th>P value</th>
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<tr>
<td>FT4 (pmol/liter)</td>
<td>1.4 ± 0.2</td>
<td>24.8 ± 1.2</td>
</tr>
<tr>
<td>TSH (mU/liter)</td>
<td>142.4 ± 10.4</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>T4 (nmol/liter)</td>
<td>0.3 ± 0.1</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>rT3 (nmol/liter)</td>
<td>0.02 ± 0.00</td>
<td>0.29 ± 0.02</td>
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Wilcoxon signed rank test. Data are presented as mean ± SE.

Results

DTC patient characteristics and thyroid hormone levels

Eleven patients were included in the DTC group (four males and seven females). Mean age (± SE) was 45.5 ± 2.1 yr. Mean duration (± SE) of TSH suppressive therapy was 5.0 ± 2.1 yr (range, 0.6–24.3 yr). Mean T4 dosage (± SE) was 197 ± 13 μg/d. Thyroid parameters are presented in Table 2.

After 4 wk of T4 withdrawal, all patients were overtly hypothyroid. After 8 wk of T4 replacement therapy, FT4, T3, and rT3 increased significantly (P = 0.003), whereas TSH decreased significantly (P = 0.003). Six patients had thyroid parameters within the reference range, whereas five patients had a TSH below the reference range with normal plasma T3 and T4 levels.

Skeletal muscle deiodinase expression and D2 activity in DTC patients

Results of the quantitative RT-PCR analysis of deiodinase mRNA levels in skeletal muscle biopsies are presented in Fig. 1A. D2 and D3 mRNA levels were present in all muscle biopsies, but there was no significant difference between the hypothyroid and T4 replacement states.

![Fig. 1. mRNA levels of D2 and D3 vs. the household gene GAPDH or cyclophilin A (×10^7) in skeletal muscle samples (mean ± SE). A. In DTC patients (n = 11) during hypothyroidism and during T4 replacement treatment. B. In healthy subjects (n = 6) after 14 and 62 h of fasting before and after insulin infusion. P < 0.05 for D2 mRNA expression in the basal state after 62 h of fasting compared with the basal state after 14 h of fasting and D2 mRNA expression after insulin infusion after 62 h of fasting compared with the basal state after 62 h of fasting.](image-url)
with 62 h of fasting. The T3/T4 (data not shown) and T3/rT3 levels were significantly lower after 14 h of fasting compared with 62 h of fasting (mean SE). Little D3-catalyzed conversion of T4 to rT3 was observed in the skeletal muscle biopsies, and this was also not different between the hypothyroid and T4 replacement states (Fig. 2A) and using the iodide release assay (data not shown). D3 mRNA measurements were not reliable due to contamination with genomic DNA.

Very little D2 activity was detected in the muscle biopsies, and it was not different between the T4 replacement and hypothyroid states (Fig. 2A). Similar results were obtained by HPLC analysis of T4 formation (Fig. 2A) and using the iodide release assay (data not shown). Little D3-catalyzed conversion of T4 to rT3 was observed in the skeletal muscle biopsies, and this was also not different between the hypothyroid and T4 replacement states (data not shown).

Fasting subject characteristics and thyroid hormone levels

Six lean healthy men were included. Subject characteristics were: age, 23 ± 1.6 yr; weight, 69.4 ± 2.2 kg after 14 h and 67.5 ± 2.2 kg after 62 h of fasting, P = 0.002; BMI, 21.2 ± 0.7 kg/m² after 14 h and 20.5 ± 0.7 kg/m² after 62 h of fasting, P = 0.001 (23).

Plasma FT4 and TSH levels were not different between 14 and 62 h of fasting (Table 3). T3 levels were significantly higher after 14 h of fasting compared with 62 h of fasting. The T3/T4 (data not shown) and T3/rT3 ratios were significantly higher after 14 h of fasting compared with 62 h of fasting.

Skeletal muscle deiodinase expression and D2 activity after 14 and 62 h of fasting

D2 mRNA levels in skeletal muscle biopsies were significantly lower after 62 h of fasting compared with 14 h of fasting in the basal state (P = 0.028) (Fig. 1B). No differences in D2 mRNA levels were observed during hyperinsulinemia after 62 h compared with 14 h of fasting.

Insulin infusion did not significantly increase D2 mRNA expression after 14 h of fasting, whereas insulin induced a significant increase in D2 mRNA levels after 62 h of fasting (P = 0.028). D3 mRNA measurements were not reliable due to contamination with genomic DNA.

D2 activity was low, but detectable, in the muscle biopsies in the basal state and after 5 h of hyperinsulinemia after both 14 and 62 h of fasting. However, no significant differences were found (Fig. 2B). Very little D3 activity was demonstrated, and also here no differences were found (data not shown).

Different household genes were used for standardization of mRNA measurements in DTC patients and healthy fasting subjects. However, their expression levels were constant in the patients and healthy subjects during the different conditions.

D2 activity, lidocaine, and possible inhibitors in muscle homogenate

Addition of increasing concentrations up to 1 mM of lidocaine did not inhibit D2 activity expressed in COS1 cells transfected with human D2 cDNA (Fig. 3A). Although a dose-dependent inhibition of D2 activity was observed after addition of increasing volumes of muscle homogenate up to 50% of the total incubation volume, remaining activity still amounted to 60% of that expressed in D2-COS1 lysates (Fig. 3B).

#### Discussion

In this study, we investigated the D2 activity and expression of D2 and D3 mRNA in skeletal muscle samples in DTC patients on and off T4 replacement therapy and in healthy subjects after 14 and 62 h of fasting and during hyperinsulinemia. Hypothyroidism induced by withdrawal of T4 substitution in thyroidectomized patients did not affect muscle D2 mRNA expression, whereas fasting for 62 h reduced muscle D2 mRNA levels compared with fasting for 14 h. Conversely, insulin increased mRNA expression after 62 h, but not after 14 h of fasting. Nonetheless, skeletal muscle D2 activities were very low and were not influenced by hypothyroidism, fasting, or insulin.

D2 activity is regulated by thyroid status at both the pre- and posttranslational level. In hypothyroidism, D2 activity is increased in different tissues predominantly by a decrease in substrate (T4)-induced degradation of D2 protein (1, 28–30). Hypothyroidism induced by withdrawal of T4 substitution in thyroidectomized patients did not affect muscle D2 mRNA expression, whereas fasting for 62 h reduced muscle D2 mRNA levels compared with fasting for 14 h. Conversely, insulin increased mRNA expression after 62 h, but not after 14 h of fasting. Nonetheless, skeletal muscle D2 activities were very low and were not influenced by hypothyroidism, fasting, or insulin.

D2 mRNA levels were observed during hyperinsulinemia after 62 h compared with 14 h of fasting.

#### Fasting subject characteristics and thyroid hormone levels

Six lean healthy men were included. Subject characteristics were: age, 23 ± 1.6 yr; weight, 69.4 ± 2.2 kg after 14 h and 67.5 ± 2.2 kg after 62 h of fasting, P = 0.002; BMI, 21.2 ± 0.7 kg/m² after 14 h and 20.5 ± 0.7 kg/m² after 62 h of fasting, P = 0.001 (23).

Plasma FT4 and TSH levels were not different between 14 and 62 h of fasting (Table 3). T3 levels were significantly higher after 14 h of fasting compared with 62 h of fasting. The T3/T4 (data not shown) and T3/rT3 ratios were significantly higher after 14 h of fasting compared with 62 h of fasting.

**TABLE 3. Thyroid hormone parameters in healthy subjects**

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<th>At baseline (n = 6)</th>
<th>During hyperinsulinemia (n = 6)</th>
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<tr>
<td></td>
<td>14-h fasting</td>
<td>62-h fasting</td>
</tr>
<tr>
<td>FT4 (pmol/liter)</td>
<td>14.6 ± 1.0</td>
<td>14.6 ± 1.3</td>
</tr>
<tr>
<td>TSH (mU/liter)</td>
<td>1.0 ± 0.2</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>T3 (nmol/liter)</td>
<td>1.6 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>rT3(nmol/liter)</td>
<td>0.24 ± 0.02</td>
<td>0.44 ± 0.02</td>
</tr>
<tr>
<td>T3/T4 ratio</td>
<td>6.9 ± 0.7</td>
<td>2.5 ± 0.2</td>
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Wilcoxon signed rank test. Data are presented as mean ± se.
Both the decrease in serum T₃ and increase in serum rT₃ may be explained by decreased D1 activity (in liver/kidney), decreased D2 activity (in muscle), or increased D3 activity (in the central nervous system) (2).

D2 mRNA levels in skeletal muscle samples were significantly lower after 62 h of fasting compared with 14 h of fasting. Serum insulin levels were also lower after 14 h than after 62 h of fasting. The decreased muscle D2 mRNA expression after 62 h of fasting was increased after insulin infusion, whereas insulin did not stimulate D2 mRNA levels after 14 h of fasting. These findings suggest that insulin regulates D2 mRNA expression mainly in the lower plasma insulin range, with median levels of 30 pmol/liter being already sufficient to maximally stimulate D2 mRNA expression.

Our findings are consistent with previous reports. In mice, fasting decreased the expression of D2 in the pituitary (41). It had been shown in rats that D2 activity and mRNA expression in BAT are up-regulated by insulin (19–21), and more so in insulin-deficient rats than in normal rats (21). Also, addition of insulin to rat brown adipocytes in vitro leads to an increase in Vₘₐₓ of D2 (20). Martinez-deMena and Obregón (19) found that this induction is not a direct effect of insulin, but that insulin improves the adrenergic stimulation of D2 activity. The exact mechanism by which fasting and hyperinsulinemia affect D2 mRNA expression in skeletal muscle is unknown. Interestingly, very recently Grozovsky et al. (22) showed a possible relationship between D2 and insulin signaling with a reduction in insulin-stimulated phosphorylation of AKT in muscle of D2-knockout mice. Whether reduced D2 expression is also responsible for the lower AKT-phosphorylation after fasting remains to be elucidated (23).

Little D2 activity was present in skeletal muscle samples after 14 and 62 h of fasting, and no difference was observed between the two conditions, in contrast with the observed changes in D2 mRNA expression. There are several possible explanations for this. The particular level of D2 mRNA expression in skeletal muscle may not result in significant D2 activity. Therefore, D2 activity in other tissues may be responsible for the decrease in serum T₃ levels during prolonged fasting. In rats, D1 activity in the thyroid and liver and D2 activity in the thyroid were decreased after fasting (42, 43), whereas D2 activity in the hypothalamus was increased (44). In other conditions where T₃ levels decrease significantly, such as acute critical illness, no D2 activity could be measured in liver and skeletal muscle biopsies (33). However, Mebis et al. (13) found low but significant skeletal muscle D2 activity during prolonged critical illness, suggesting an adaptation to the low T₃ levels. However, such an adaptation was not observed in our hypothyroid subjects 4 wk after T₄ withdrawal.

It is unlikely that the local anesthetic used for the sample collection could have influenced D2 activity because we found no effect on D2 activity in D2-COS1 cells with increasing lidocaine concentrations. However, we cannot exclude a local effect of lidocaine resulting in down-regulation of D2 activity. On the other hand, Mebis et al. (13) reported no differences in D2 expression and D2 activity in muscle samples taken under local anesthetics or during laparotomy. Addition of skeletal muscle homogenate up to 50% of the total incubation volume, which is also the concentration of homogenate tested for muscle D2 ac-
tivity, maximally decreased D2 activity in D2-COS1 cell lysates by only 40%. It is therefore unlikely that inhibiting factors present in muscle homogenates are responsible for the very low D2 activities we found in skeletal muscle biopsies.

Recent findings show that D3 mRNA and activity (catalyzing T₄ to rT₃ deiodination and T₃ degradation) may be increased in muscle and liver of patients hospitalized in the intensive care unit (45). However, we found no change in D3 activity in skeletal muscle during fasting or hyperinsulinemia.

In summary, no differences were observed in the expression of skeletal muscle D2 mRNA between hypothyroidism and T₄ treatment, although a robust decrease was observed after 62 h of fasting. Moreover, insulin restored D2 mRNA expression after 62 h of fasting. Little D2 activity was measured in skeletal muscle samples, and no differences were observed between hypothyroidism and T₄ treatment or after 14 and 62 h of fasting. Our results therefore imply that skeletal muscle D2 mRNA expression is modulated by fasting and insulin, but not by hypothyroidism or T₄ treatment. Whereas the support for a metabolic role for D2 in human muscle is growing, the lack of effect of changes in D2 mRNA on already low D2 activity questions the importance of a role for D2 activity in extrathyroidal T₃ production in human skeletal muscle.

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Disclosure Summary: The authors have nothing to declare.

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