

# Type 3 Iodothyronine Deiodinase Is Highly Expressed in the Human Uteroplacental Unit and in Fetal Epithelium

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Type 3 iodothyronine deiodinase (D3) is the major physiologic inactivator of thyroid hormone. This selenoenzyme, previously identified in human placenta and brain, catalyzes the inner-ring deiodination of  $T_4$  to reverse  $T_3$  and  $T_3$  to 3,3'-diiodothyronine, both of which are biologically inactive. We analyzed D3 expression in several human adult and fetal tissues by immunohistochemistry and correlated the results with D3 activity assays where possible. High D3 expression was present in the placental syncytiotrophoblasts and cytotrophoblasts, endothelium of fetal vessels, and maternal decidua. D3 was also present at other

sites of maternal-fetal interface, including the umbilical arteries and vein and the fetal respiratory, digestive, and urinary tract epithelium. Surprisingly, D3 was also present in the endometrial glands of nonpregnant human uteri, and endometrial activity approximated that of term placenta. The presence of D3 at maternal-fetal interfaces is consistent with its role in modulating the thyroid status of the human fetus and its expression in endometrium suggests that local regulation of thyroid status is important in implantation. (*J Clin Endocrinol Metab* 88: 1384–1388, 2003)

HUMAN PREGNANCY IS characterized by maternal-to-fetal gradients of free  $T_4$  and  $T_3$ , which have been attributed to the expression of D3 in placenta (1–5). Recent studies have also identified type 3 iodothyronine deiodinase (D3) mRNA and activity in the pregnant rat uterus and in a variety of mammalian fetal structures, including the cerebrum, cerebellum, skin, liver, kidney, and intestine (6–8).

Despite these studies, the specific cell types of the human fetoplacental unit that express D3 are unknown. We applied previously described immunohistochemical techniques to identify D3-expressing cells in the human placenta, uterus, and fetus (9). D3 is expressed in the syncytio- and cytotrophoblasts, endothelium of the placental and umbilical cord vessels, uterine decidua, epithelial cells of the human fetus, and nonpregnant human endometrium. These novel results suggest that the local modulation of thyroid status is important at all stages of human reproduction.

## Materials and Methods

### *Tissue preparation and D3 assays*

Studies of human tissues were approved by the Investigative Review Board of the Brigham and Women's Hospital. Surgical and autopsy specimens were snap frozen in liquid nitrogen and homogenized in approximately five volumes of 0.25 M sucrose, 10 mM dithiothreitol in phosphate EDTA buffer (pH 6.9). D3 activity was assayed as previously described (10). In brief, after incubation at 37°C for 60 min, reactions were stopped by the addition of ice-cold methanol and centrifuged. The products of deiodination were identified and quantitated by HPLC as described by Richard *et al.* (8). Maximal velocity ( $V_{max}$ ) and Michaelis constants ( $K_m$ ) are expressed as mean values  $\pm$  SE.

Abbreviations: D3, Type 3 iodothyronine deiodinase; HEK, human embryonic kidney;  $K_m$ , Michaelis constant; PTU, propylthiouracil;  $V_{max}$ , maximal velocity.

### *Immunohistochemistry*

The primary D3 antibody, D3–18, was prepared in rabbits using a peptide deduced from the *hDio3* sequence (53-KPEPELVNSEGEEVP-68) and then affinity purified as previously described (9, 11). Four-micron sections were cut from formalin-fixed, paraffin-embedded tissue blocks and microwaved at 93°C in 10 mM citrate buffer for antigen retrieval. Sections were incubated with primary D3 antibody at 1:100 dilution and then processed with a Vectastain Elite ABC immunoperoxidase kit (Vector Laboratories, Inc., Burlingame, CA). 3,3'-Diaminobenzidine chromogen was used to localize peroxidase activity, and slides were counterstained with 1% Gill's hematoxylin. Isotype controls were performed on all specimens to confirm specificity using an equivalent concentration of rabbit IgG (Vector Laboratories) in place of the primary antibody.

To confirm the specificity of D3–18 antibody staining, human embryonic kidney (HEK) 293 cells transfected with a plasmid expressing hD3 were pelleted, fixed, and stained using the above methods. These were subjected to immunohistochemical staining simultaneously with untransfected HEK 293 cells and HEK 293 cells transfected with empty expression vector. Although these cells showed no immunostaining, HEK cells that were transfected with plasmid expressing hD3 showed strong immunostaining of approximately 15–20% of cells, reflecting the typical efficiency of transient transfection with calcium phosphate-DNA precipitates (data not shown).

## Results

### *D3 is expressed in the decidua, syncytiotrophoblast layer, cytotrophoblasts, and fetal microvessels of the human uterus and placenta*

Immunohistochemistry was performed on samples of human placenta from each trimester. D3 was present in the maternal decidua, syncytiotrophoblast layer, cytotrophoblasts, and fetal endothelium of the chorionic villi (Fig. 1). The specificity of the D3–18 antibody was demonstrated by blockade of placental immunostaining after preincubation with  $10^{-5}$  M isologous peptide (data not shown). For all specimens examined, isotype controls were negative. No

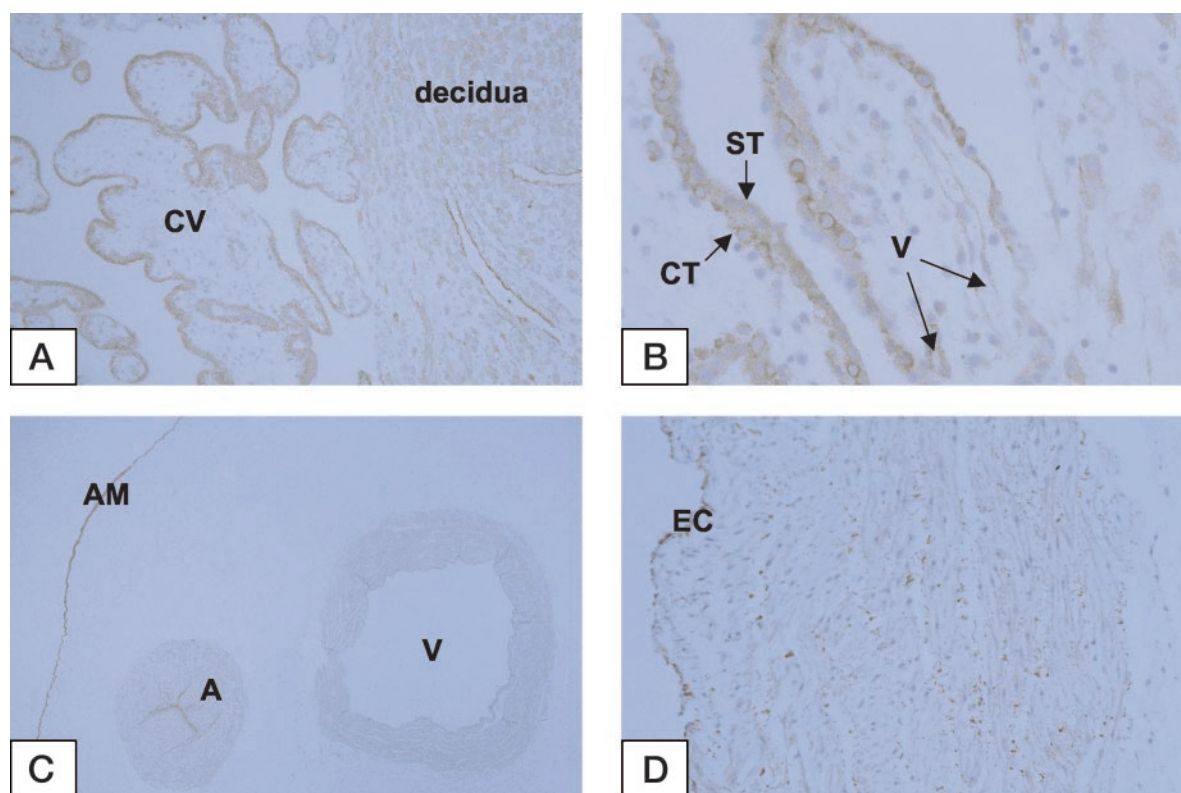


FIG. 1. D3 immunohistochemical staining of the uterus, placenta, and umbilical cord. A and B, Low ( $\times 100$ ) and high ( $\times 400$ ) power of D3 antibody staining in first-trimester placenta (from wk 13 of gestation). C and D, Low ( $\times 20$ ) and high ( $\times 200$ ) power of D3 antibody staining in term umbilical cord. Intense D3 staining is present in the maternal decidua as well as in the syncytiotrophoblasts (ST), cytotrophoblasts (CT), and endothelial cells of the fetal microvessels (V) in the chorionic villi (CV). Umbilical cord D3 is localized to the umbilical arteries (A), vein (V), and amniotic sheath (AM). In D, staining is strongest in the endothelial cells (EC) of the umbilical vessels with weaker staining of the perivascular myocytes.

obvious differences in the cellular distribution or intensity of placental D3 staining were noted throughout gestation, other than the normal fragmentation of the cytotrophoblastic layer characteristic of the third trimester.

#### *D3 is expressed in the blood vessels and amnion sheath of the umbilical cord*

Immunohistochemistry of term umbilical cord revealed strong D3 staining of the endothelial cells and the perivascular myocytes of the umbilical arteries and vein. The amnion sheath that forms the epithelial covering of the cord also stained for D3, but the mucoid connective tissue surrounding the umbilical blood vessels did not (Fig. 1).

To support this immunohistochemical finding, fresh umbilical cord specimens were assayed for D3 activity. Six umbilical cords were collected from uncomplicated, full-term pregnancies. All showed propylthiouracil (PTU)-resistant inner-ring deiodinase activity of T<sub>3</sub> with a velocity of  $3.3 \pm 0.6$  fmol/min/mg in the presence of 10 nM T<sub>3</sub>.

#### *D3 is expressed in epithelial cells of the human fetus*

The epithelium of the fetal tracheobronchial tree, small intestine, urothelium, and skin all stained positively for D3 (Fig. 2). This was present in the respiratory epithelium from the trachea down to the alveoli. The epithelial layer of the intestinal villi was also strongly positive. Urothelial staining was visualized from the level of the ureter down to the

bladder. Staining of fetal skin localized to the superficial layers of the epidermis and to the endothelial cells of the dermal microvessels. In all cases, the connective tissues supporting the epithelium did not stain, providing an internal negative control. In the small intestine and bladder, weak staining of the muscularis propria was also noted.

#### *D3 is expressed in the endometrial glands of nonpregnant uterus*

To analyze the time of appearance of D3 expression during gestation, we examined uterine tissue from nonpregnant women. Surprisingly, the endometrial glands stained strongly for D3. To determine whether this expression was influenced by the menstrual cycle, archived paraffin-embedded curettage specimens, representing the various stages of the menstrual cycle, were examined. The endometrial glands stained strongly at all stages with no discernible difference in the intensity or cellular distribution of D3 observed through the menstrual cycle. Weaker staining was also present in the myometrium but was essentially limited to the endothelial cells of the myometrial vasculature (Fig. 3). The other uterine stromal elements did not stain.

To corroborate these results, fresh surgical specimens of benign, nonpregnant uteri removed for fibroid disease were prospectively collected and assessed for D3 activity. Eleven samples were collected from patients ranging in age from 30–68 yr. All uterine samples expressed D3 activity, as ev-

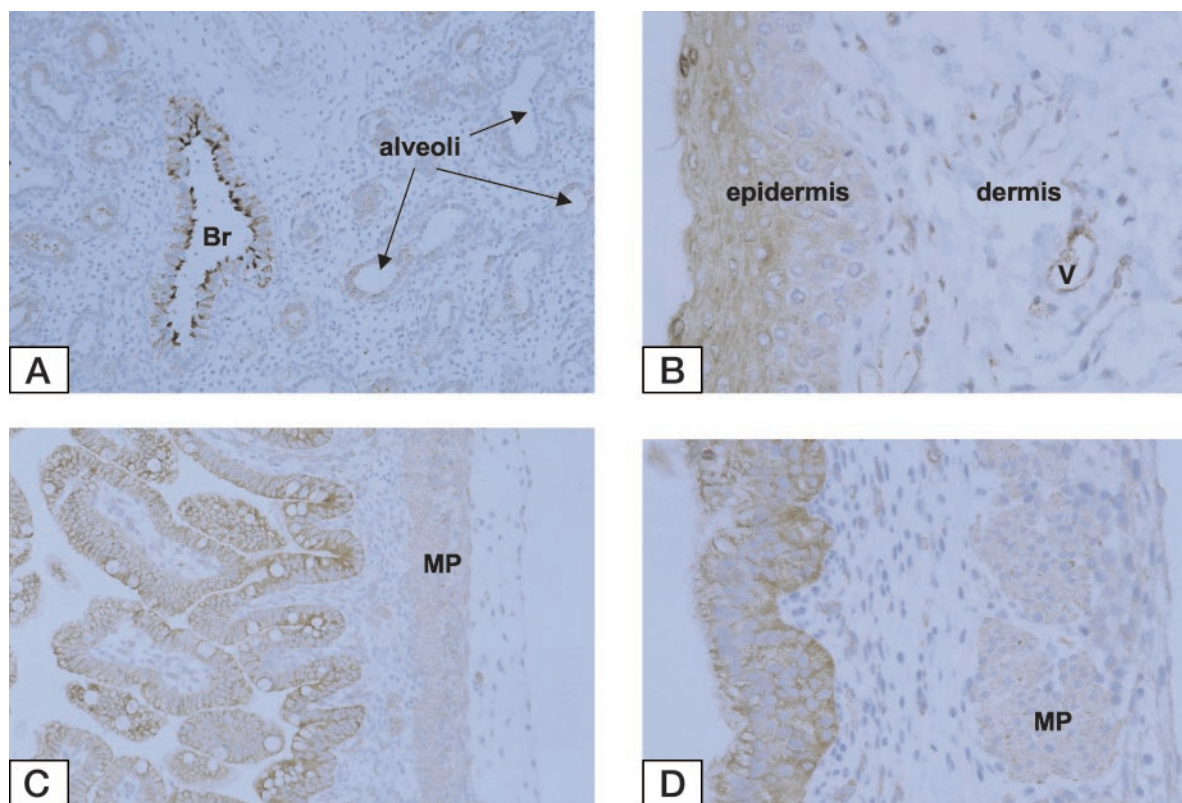


FIG. 2. D3 is highly expressed in the fetal epithelium. The D3 antibody stains the epithelial surfaces of the tracheobronchial tree (A), skin (B), small intestine (C), and urinary tract (D). The specimens shown are from a 19-wk fetus (A), a 30-wk fetus (B), and a 13-wk fetus (C and D). A and C are low ( $\times 200$ ) power, and B and D are high ( $\times 400$ ) power. In A, epithelial staining is present from the trachea to the bronchiole (Br) and also in the lining of the alveolar air spaces. In B, staining is strongest in the superficial epidermis with small vessels (V) staining in the dermal layer. In C and D, staining is strongest in the epithelial cells with weaker staining of the muscularis propria (MP).

identified by a low  $K_m$ ,  $1.6 \pm 0.3$  nM  $T_3$ , and resistance to PTU inhibition. The mean  $V_{max}$  of these samples was  $5.3 \pm 2.2$  fmol/min/mg. In two additional specimens, the endometrial layer was isolated by scraping the uterine cavity with a scalpel blade, and the D3 activity was compared with that of myometrium from the same specimen. D3 activity was confirmed by a low  $K_m$ ,  $1.8 \pm 0.6$  nM  $T_3$ , and resistance to 1 mM PTU. The  $V_{max}$  values of the endometrial specimens (159 and 66 fmol/min/mg) were greater than that of a term placenta (45 fmol/min/mg) assayed as a positive control. Consistent with the immunostaining shown in Fig. 3, endometrial D3 activity was 62- and 41-fold higher than that of the corresponding myometrium. (Fig. 4.)

### Discussion

In this study, we used immunohistochemistry to identify D3-expressing cell types. This technique avoids the need for tissue homogenization, in which protein from cells with high D3 activity is diluted with that from non-D3-expressing cells. D3 is expressed in multiple endothelial and epithelial cells in the uteroplacental unit and fetus. The specificity of the staining was confirmed by isotype controls and, when feasible, D3 activity assays.

The placental membrane is a composite of extrafetal tissues separating the maternal and fetal circulations. Early in pregnancy, it consists of four components: the syncytiotrophoblasts, cytotrophoblasts, loose connective tissue consti-

tuting the core of each villus, and endothelium of the fetal capillaries. After the 20th wk of gestation, the cytotrophoblast layer becomes fragmented, such that three rather than four components remain. D3 expression in the human placenta has been recognized for many years, and it effectively blocks the maternal-to-fetal transfer of  $T_4$  as demonstrated by direct studies of human placental lobules (5). Consistent with this function, D3 is highly expressed in the syncytiotrophoblast and cytotrophoblast layers as well as in the fetal endothelium of the chorionic villi. Strong D3 staining is also seen in the maternal decidua of the human placenta and in the amnion sheath of the umbilical cord, as might be expected from positive *in situ* hybridization results in pregnant rat uterus and deiodination assays in human fetal membranes (6, 12).

In addition to the placenta, amniotic fluid is a second potential pathway for the entry of maternal  $T_4$ . The human fetus swallows up to 400 ml amniotic fluid per day, which is absorbed into the fetal circulation and excreted by the kidneys (13). Fluid transfer also occurs through the amniochorionic membrane, umbilical cord, and fetal respiratory epithelium. Despite these potential sites for exchange, the concentration of free  $T_4$  in amniotic fluid is higher than in maternal or fetal serum (2). This gradient indicates that tissues in addition to placenta also limit the transfer of  $T_4$  to the fetus. This can now be explained by D3 expression in the umbilical vessels and respiratory, intestinal, skin, and uri-

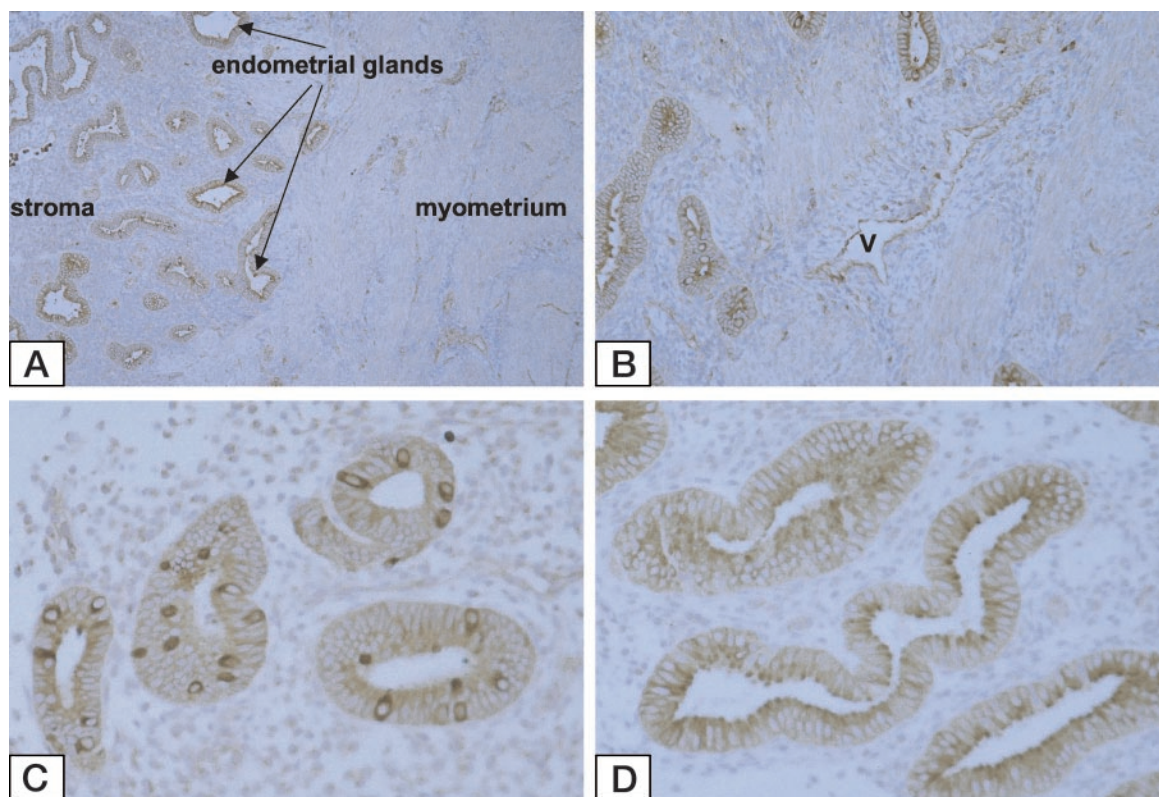


FIG. 3. D3 expression in human endometrium. A and B, Low ( $\times 40$ ) and high ( $\times 100$ ) power of D3 antibody staining in nonpregnant uterus. Strong D3 staining is present in the endometrial glands. Weaker staining is also present in the myometrium, particularly in the endothelial cells of the myometrial blood vessels (V). The stromal tissue surrounding the endometrial glands does not stain. C and D, high ( $\times 200$ ) power of D3 antibody staining in curettaged specimens of proliferative (C) and secretory (D) endometrium.

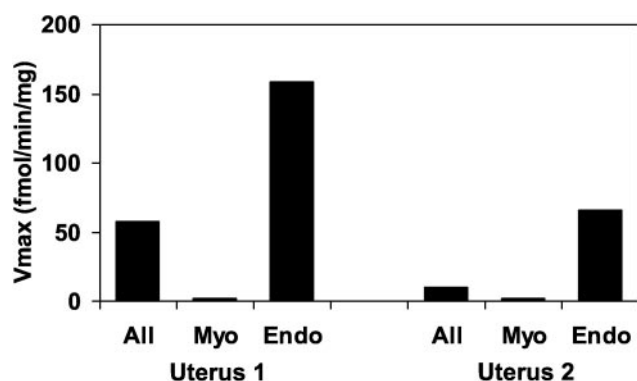


FIG. 4. Uterine D3 activity localizes to the endometrial glands. The endometrial (Endo) and myometrial (Myo) layers of two nonpregnant human uteri were dissected and assayed for D3 activity in the presence of 1 mM PTU. The values shown are Vmax as determined by Lineweaver-Burk analysis.

nary tract epithelium of the fetus. D3 is also highly expressed in the skin of the newborn rat (7).

The precise control of local thyroid hormone concentrations is critical to embryonic development. The coordinated expression of  $T_4$  activating and  $T_4$  and  $T_3$  inactivating iodothyronine deiodinases is required for amphibian morphogenesis and rodent studies have implied a similar function in mammalian embryos. Fetal D3 activity is present in rat and human liver and rat skin, brain, and intestine (7, 8, 14–16). It has been postulated that the expression of D3 in fetal

structures protects them from the temporally inappropriate action of thyroid hormones. This concept is supported in humans by the adverse effects of fetal hyperthyroidism because of maternal Graves' disease. Affected infants may have irreversible neurologic and skeletal disease because of premature synostosis and disordered neuronal differentiation (17–20).

The finding of high D3 activity in the nonpregnant human uterus was unexpected and contrasts with the low D3 activity reported in nonpregnant rat uterus (6). This apparent discrepancy could be related to the highly focal localization of D3 expression in the endometrial glands. The human uteri we tested had widely variable D3 activity not related to menstrual cycle, but the endometrial homogenates had Vmax values that were greater than that of term placenta. No obvious cyclical change in endometrial D3 expression was discernible by immunohistochemistry, but this may reflect limitations of this technique as a quantitative tool. However, an increase in uterine D3 during early gestation could help explain the 50% increase in  $T_4$  requirements typically observed during the first trimester of pregnancy (21, 22).

These studies also provide the first evidence of D3 expression in normal human endothelium. Previously endothelial D3 activity had been identified only in infantile hemangiomas and one hemangioendothelioma (9, 10). Many similarities exist in the cellular expression profiles of infantile hemangioma endothelium and the fetal endothelium of the chorionic villi (23, 24). Our results indicate that D3 is yet

another protein shared between these two cell types. In keeping with the concept of endothelial D3 expression, differentiating cytotrophoblasts transform their adhesion receptor phenotype to resemble the endothelial cells they replace during uterine invasion, and the syncytiotrophoblast and cytotrophoblast layers both function in the placenta as a type of modified vasculature as they surround the lacunae of maternal blood (25, 26).

D3 catalyzes the bulk of  $T_4$  to reverse  $T_3$  and  $T_3$  to  $3,3'$ - $T_2$  conversion in humans, and yet the only previously recognized anatomical location in the nonpregnant human is the central nervous system (1, 11, 27). The identification of D3 expression in the endothelial cells of the fetoplacental unit shown here also raises the possibility that other endothelial cells can express this enzyme. This could help account for the major physiological role of D3 in human thyroid hormone metabolism beyond that in reproduction and fetal development. The consumptive hypothyroidism associated with the expression of D3 in the endothelium of infantile hemangiomas provides ample evidence of its potency in metabolizing circulating  $T_3$  and  $T_4$  (9, 10).

### Acknowledgments

Received August 14, 2002. Accepted December 11, 2002.

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This work was supported by NIH Grants DK60494 and DK44128, the Lawson Wilkins Abbott Clinical Scholar Award from the Lawson Wilkins Pediatric Endocrine Society, the Charles A. King Trust Postdoctoral Research Fellowship Award from the Medical Foundation (Boston, MA), the Charles A. Janeway Child Health Research Center Award from the Child Health Research Center of Children's Hospital Boston, and the Doris Duke Clinical Scientist Development Award from the Doris Duke Charitable Foundation.

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