Differential Triiodothyronine Responsiveness and Transport by Human Cytotrophoblasts from Normal and Growth-Restricted Pregnancies

E. Vasilopoulou, L. S. Loubière, A. Martin-Santos, C. J. McCabe, J. A. Franklyn, M. D. Kilby, and S.-Y. Chan

School of Clinical and Experimental Medicine, College of Medical and Dental Sciences, University of Birmingham, Birmingham B15 2TT, United Kingdom

Context: Abnormal placentation in human pregnancy is associated with intrauterine fetal growth restriction (IUGR). Our group has previously reported the association between severe IUGR, lower fetal circulating concentrations of thyroid hormones (THs), and altered expression of TH receptors and TH transporters within human placental villi. We postulate that altered TH bioavailability to trophoblasts may contribute to the pathogenesis of IUGR.

Design and Objective: Cytotrophoblasts were isolated from normal and IUGR human placentae to compare their responsiveness to T3 and their capability for T3 transport.

Results: Compared with normal cytotrophoblasts, the viability of IUGR cytotrophoblasts (assessed by methyltetrazoleum assay) was significantly reduced ($P < 0.001$), whereas apoptosis (assessed using caspase 3/7 activity and M30 immunoreactivity) was significantly increased after T3 treatment for 48 h ($P < 0.001$ and $P < 0.01$, respectively). The secretion of human chorionic gonadotropin was significantly increased by IUGR cytotrophoblasts compared with normal cytotrophoblasts ($P < 0.001$), independently of T3 treatment. Net transport of $[^{125}]$T3 was 20% higher by IUGR cytotrophoblasts compared with normal cytotrophoblasts ($P < 0.001$), and this was accompanied by a 2-fold increase in the protein expression of the TH transporter, monocarboxylate transporter 8, as assessed by Western immunoblotting ($P < 0.01$).

Conclusions: IUGR cytotrophoblasts demonstrate altered responsiveness to T3 with significant effects on cell survival and apoptosis compared with normal cytotrophoblasts. Increased monocarboxylate transporter 8 expression and intracellular T3 accumulation may contribute to the altered T3 responsiveness of IUGR cytotrophoblasts. (J Clin Endocrinol Metab 95: 4762–4770, 2010)
Untreated maternal hyperthyroidism has been associated with complications of malplacentation, including IUGR, placental abruption, and preeclampsia (16), whereas maternal subclinical hypothyroidism has been associated with increased risks of miscarriage, placental abruption, and preterm delivery (17, 18), which suggests some influence of maternal thyroid hormones (THs) on human placentation. In vitro, T₃, the active TH ligand, increases the invasive capability of first trimester human extravillous trophoblasts (19) and suppresses apoptosis in this cell type (20).

Although circulating concentrations of THs are the major determinants of cellular TH supply, there are several prerequisites for effective TH action, including TH transport into cells and prereceptor regulation of T₂, by iodothyronine deiodinases and T₃ binding to nuclear TH receptors (TRs) that regulate the expression of TH-responsive genes (21). Our group has previously reported changes in the mechanisms regulating TH action in placentae from severe IUGR pregnancies delivered in the early third trimester. The protein expression of the TR isoforms TRα1, TRα2, and TRβ1 is increased in placental villi from severe IUGR pregnancies (22) with no significant change in the activities of placental deiodinase types 2 and 3 (23). In addition, the expression of the TH transporter monocarboxylate transporter 8 (MCT8; official symbol, SLC16A2) is increased, whereas the expression of MCT10 (SLC16A10) is decreased in the villous placenta with severe IUGR (24, 25). Using percutaneous in utero fetal blood sampling, we have also reported that the circulating concentrations of free T₄ and free T₃ are significantly reduced in severely growth restricted fetuses (22).

We hypothesize that the reported changes in the expression of TRs and TH transporters in IUGR placentae may be associated with altered trophoblast sensitivity to THs in IUGR pregnancies. In this study, primary cultures of cytotrophoblasts isolated from third trimester placentae from uncomplicated pregnancies (normal cytotrophoblasts) or from pregnancies complicated by IUGR (IUGR cytotrophoblasts) were used to assess differences in T₃ responsiveness and to investigate whether changes in TH transport may account for such differences.

**Subjects and Methods**

**Sample collection**

Human placentae from normal (n = 27) and IUGR (n = 14) pregnancies were collected with informed written consent and local research ethics committee approval after elective delivery by cesarean section. All were delivered after 35 completed weeks of gestation, as determined by a first trimester ultrasound scan of crown-rump length. The IUGR cases were diagnosed prospectively using ultrasound and had at least two of the following characteristics: 1) abdominal circumference growth velocity of less than 1.5 SD values over 14 d; 2) oligohydramnios, defined as maximum pool depth of 10th centile or less for gestation; and 4) absent or increased resistance index in the end diastolic flow velocity of the umbilical artery Doppler velocity waveform. The IUGR fetuses were not known to have abnormal karyotypes, and none of the pregnancies was complicated by maternal hypertension or thyroid disorders.

**Human chorionic gonadotropin (hCG) secretion**

Cytotrophoblasts were seeded in 12-well plates (1.5 × 10⁶ cells/well) and 18 h after isolation were treated with 0, 10, or 100 nM T₃ for 48 h. Cell survival was assessed by four replicates using the methyltetrazoleum (MTT; Sigma-Aldrich) assay as described previously (26). Cell apoptosis was assessed in triplicate using the luminescence-based caspase 3/7 activity assay (Promega, Southampton, UK) as described previously (29). The results were normalized to the values obtained with no T₃ treatment (0 nM) within each experiment. In addition, apoptosis in response to T₃ was assessed with immunofluorescent staining for the apoptotic marker, M30 (30). Cytotrophoblasts were seeded in duplicate in 24-well plates (7.5 × 10⁵ cells/well) and 48 h after T₃ treatment were fixed and permeabilized with 100% methanol. Cytotrophoblasts were probed with primary antibody against M30 (1:50; Roche, Burgess Hill, UK), followed by Alexa Fluor 488-conjugated secondary antibody (1:250; Invitrogen) and the nuclear stain, Hoechst 33258 (1:1000; Sigma-Aldrich). M30 staining was assessed by a researcher blinded to placentae type and treatment. The number of nuclei with M30 perinuclear staining was expressed as a percentage of the total number of nuclei. The results were normalized within each experiment to the average percentage of M30-positive nuclei with no T₃ treatment (0 nM).

**Assessment of cell survival and apoptosis**

Cytotrophoblasts were seeded in 96-well plates (3 × 10⁵ cells/well) and 18 h after isolation were treated with 0, 1, 10, or 100 nM T₃ for 48 h. Cell survival was assessed by four replicates using the methyltetrazoleum (MTT; Sigma-Aldrich) assay as described previously (26). Cell apoptosis was assessed in triplicate using the luminescence-based caspase 3/7 activity assay (Promega, Southampton, UK) as described previously (29). The results were normalized to the values obtained with no T₃ treatment (0 nM) within each experiment. In addition, apoptosis in response to T₃ was assessed with immunofluorescent staining for the apoptotic marker, M30 (30). Cytotrophoblasts were seeded in duplicate in 24-well plates (7.5 × 10⁵ cells/well) and 48 h after T₃ treatment were fixed and permeabilized with 100% methanol. Cytotrophoblasts were probed with primary antibody against M30 (1:50; Roche, Burgess Hill, UK), followed by Alexa Fluor 488-conjugated secondary antibody (1:250; Invitrogen) and the nuclear stain, Hoechst 33258 (1:1000; Sigma-Aldrich). M30 staining was assessed by a researcher blinded to placentae type and treatment. The number of nuclei with M30 perinuclear staining was expressed as a percentage of the total number of nuclei. The results were normalized within each experiment to the average percentage of M30-positive nuclei with no T₃ treatment (0 nM).

**T₃ uptake and efflux**

**T₃ uptake**

Cytotrophoblasts were cultured in duplicate in 10% FCS-supplemented medium in 12-well plates for 66 h (1.5 × 10⁶ sound, less than the 10th centile for gestation; 2) abdominal circumference growth velocity of less than 1.5 SD values over 14 d; 3) oligohydramnios, defined as maximum pool depth of 10th centile or less for gestation; and 4) absent or increased resistance index in the end diastolic flow velocity of the umbilical artery Doppler velocity waveform. The IUGR fetuses were not known to have abnormal karyotypes, and none of the pregnancies was complicated by maternal hypertension or thyroid disorders.
cells/well). After incubation for 0, 5, 10, or 30 min with serum free medium (SFM) supplemented with 1 nM T₃ containing approximately 2 × 10⁵ cpm of [¹²⁵I]T₃ (Perkin-Elmer, Wellesley, MA), cytotrophoblasts were rapidly washed three times with ice-cold SFM with 0.1% BSA and were lysed with 2% sodium dodecyl sulfate (SDS). The radioactivity in the cell lysates (cellular radioactivity) was measured using a γ-counter and expressed as a percentage of the total radioactivity in the incubation media, which was added to the cells initially (31) (Supplemental Fig. 1, published on The Endocrine Society’s Journals Online web site at http://cem.endojournals.org).

**T₃ efflux**

Cytotrophoblasts were incubated in duplicate with SFM supplemented with 1 nM T₃ containing [¹²⁵I]T₃ (2 × 10⁵ cpm/0.5 ml) for 10 min. Cytotrophoblasts were briefly washed with SFM with 0.1% BSA and incubated in SFM without T₃ (efflux medium) for 0, 1, 2, 5, or 10 min. The medium was then removed, and cytotrophoblasts were lysed with 2% SDS. The proportion of the radioactivity that was retained in the cell lysates compared with that added to the cells initially was calculated. T₃ efflux was expressed as a percentage of the cellular radioactivity normalized to time 0 (just before the addition of efflux medium) (31).

**Quantitative TaqMan PCR**

Cytotrophoblasts were cultured in 35-mm² tissue culture dishes (3 × 10⁶ cells/dish) in 10% FCS-supplemented medium. Total RNA was extracted at 18 or 66 h after culture with TRI reagent (Ambion, Warrington, UK) following the manufacturer’s guidelines. RNA (1 μg) was reverse-transcribed using avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI) in a total reaction volume of 20 μl following the manufacturer’s guidelines. Expression of mRNA encoding TH transporters MCT8 and MCT10; the system L-amino acid transporters LAT1 (SLC7A5), LAT2 (SLC7A8), CD98 (SLC3A2); and the organic anion-transporting polypeptides OATP1A2 (SLCO1A2) or OATP4A1 (SLCO4A1) was determined and normalized to the expression of the housekeeping gene, 18S, as an internal control following the manufacturer’s guidelines. Expression of TH transporters MCT8 and MCT10 was assessed using TaqMan PCR. The relative quantification of each gene was expressed as a percentage of the mean gene expression in normal cytotrophoblasts at 18 h after culture that was assigned the arbitrary value of 1.

**MCT8 and MCT10 antibody production and Western immunoblotting**

Rabbit polyclonal antisera for human MCT8 (Ab4790; Sigma-Genosys, Haverhill, UK) and MCT10 (Ab2198; Charles River Laboratories, Kisslegg, Germany) were raised against synthetic polypeptides comprising amino acids 79-92 (SQASEEAKGWQEA) and 503-515 (SSGMFKKESDSII), respectively, each conjugated to keyhole limpet hemocyanin. Antisera from final bleeds were affinity-purified and their specificity confirmed (Fig. 1).

Protein was extracted from IUGR and normal cytotrophoblasts cultured for 18 or 66 h in 10% FCS-supplemented medium using 2% SDS, and Western immunoblotting was performed as previously described (25). Briefly, protein (30 μg) was denatured

![FIG. 1. Western immunoblotting confirming the specificity of polyclonal antibodies to MCT8 and MCT10. A, MCT8 4790 antibody. Whole cell protein extracts of MCT8-null JEG3 cells transfected with either vector only (VO) or a plasmid encoding human MCT8 and homogenates of term placenta tissue (Term PL) expressing endogenous MCT8 were probed with the MCT8 antibody (1.6 μg/ml) after preincubation with or without the blocking peptide (BP). B, MCT10 2198 antibody. Whole cell protein extracts of HTR8/SVNeo cells (with very low endogenous MCT10 mRNA expression) transfected with either VO or a plasmid encoding human MCT10 and extracts from normal cytotrophoblasts (CTs) that were cultured for 18 h were probed with the MCT10 antibody (5.2 μg/ml) after preincubation with or without the blocking peptide (BP). Bands of approximately 60 kDa for MCT8 (A) and approximately 50 kDa for MCT10 (B) are seen, consistent with their predicted molecular weights, respectively. Immunoreactivity for β-actin was used to assess protein loading.](https://www.jcem.org/content/95/10/4762/F1)

(1 h at room temperature) in Laemmli buffer (Bio-Rad, Hertfordshire, UK) with 350 mM dithiothreitol, separated by electrophoresis in 8% SDS-PAGE gels, and blotted onto nitrocellulose membranes. The blots were incubated with the MCT8 (3.2 μg/ml) or the MCT10 (5.2 μg/ml) antibody followed by secondary horseradish peroxidase-conjugated antibody (1:2000; Dako, Glostrup, Denmark). Antigen-antibody complexes were visualized using the ECL+ chemiluminescence detection system (GE Healthcare, St. Giles, UK). Expression of β-actin was determined to assess protein loading.

**Statistical analysis**

Data were analyzed using the Minitab statistical software (version 15; Minitab Inc., State College, PA). For demographic data, the Mann-Whitney U test was used to compare continuous variables, and the Fisher’s exact test was used to analyze contingency tables. For other data sets, ANOVA was performed using the general linear model followed by Tukey all pairwise multiple comparison *post hoc* tests to assess differences between individual groups. Residuals (differences between the observed values and the predicted values by the general linear model) for all data sets passed the normality test as determined before statistical analysis. For all tests, significance was taken as P < 0.05.
Cytotrophoblast apoptosis in response to T3

IUGR group (age, and fetal sex (32), was also significantly lower in the counts for parity, ethnicity, maternal BMI, gestational was calculated using customized growth charts and ac-

The birth weight percentile, which was calculated using customized growth charts and accounts for parity, ethnicity, maternal BMI, gestational age, and fetal sex (32), was also significantly lower in the IUGR group (P < 0.001), with a median of the 2nd percentile compared with the 53rd percentile for the normal group.

Survival of cytotrophoblasts in response to T3

Because cytotrophoblasts do not proliferate in vitro, the MTT assay was used as a measure of cell survival. Overall, IUGR cytotrophoblasts survived less compared with normal cytotrophoblasts in response to T3 treatment (Fig. 2A; P < 0.001). Post hoc analysis revealed that this difference was significant when the cells were treated with 1 nM T3 (20% reduction; P < 0.01). There was no difference in the survival of normal and IUGR cytotrophoblasts in the absence of T3 (data not shown).

Cytotrophoblast apoptosis in response to T3

We then assessed whether the effect of T3 on cytotrophoblast survival was mediated via increased apoptosis in IUGR cytotrophoblasts. Caspase 3/7 activity after T3 treatment was increased in IUGR compared with normal cytotrophoblasts (Fig. 2B; P < 0.001). Similar to what was observed with cytotrophoblast survival, the difference in caspase 3/7 activity between normal and IUGR cytotrophoblasts was particularly significant after treatment with 1 or 100 nM T3 (27% increase for both; P < 0.01). The T3-mediated increase in apoptosis of IUGR cytotrophoblasts compared with normal cytotrophoblasts was also confirmed by immunostaining for M30 (Fig. 2C), which demonstrated a highly statistically significant difference between normal and IUGR cytotrophoblasts overall (P < 0.01). The greatest increase in apoptosis of IUGR cytotrophoblasts compared with normal cytotrophoblasts occurred after treatment with 100 nM T3 (53% increase), and post hoc tests found this to be just close to significance (P = 0.058). A comparison of apoptosis in normal and IUGR cytotrophoblasts in the absence of T3 revealed no significant differences (data not shown).

hCG secretion from cytotrophoblasts in response to T3

Overall, the rise in hCG secretion over time was higher in IUGR compared with normal cytotrophoblasts (P < 0.001; Fig. 3). Post hoc tests demonstrated that this effect was significant in both the absence (P < 0.001) and the presence (P < 0.01) of T3.

T3 transport by cytotrophoblasts

These results suggested that T3 affects the behavior of cytotrophoblasts isolated from IUGR but not from normal placentae with respect to survival and apoptosis. This effect could be mediated via differences in intracellular availability of T3. To explore this possibility, we assessed T3 transport by IUGR or normal cytotrophoblasts maintained in culture for 66 h. T3 uptake was higher by IUGR compared with normal cytotrophoblasts (P < 0.001; Fig. 4A). Post hoc tests demonstrated that this difference was
only statistically significant after incubation with $^{125}$I-T3 for 30 min (23% increase; $P < 0.001$), indicating that there is an increase in net T3 transport by the cells rather than T3 uptake per se. In contrast, T3 efflux over a period of 10 min was similar between normal and IUGR cytotrophoblasts (Fig. 4B). These results therefore suggest that intracellular accumulation of T3 is higher in IUGR compared with normal cytotrophoblasts.

FIG. 2. Effect of T3 on the survival and apoptosis of normal and IUGR cytotrophoblasts. Survival and apoptosis were assessed after 48 h of treatment with 0, 1, 10, or 100 nM T3. Within each experiment, results were compared with that after no T3 treatment (0 nM), which was given an arbitrary value of 100%. A, Cytotrophoblast survival assessed using the MTT assay (normal, $n = 9$; IUGR, $n = 5$). B, Apoptosis assessed using the caspase 3/7 activity assay (normal, $n = 9$; IUGR, $n = 5$). C, Apoptosis assessed by immunofluorescent staining for M30 (normal, $n = 4$; IUGR, $n = 3$). **, $P < 0.01$; ***, $P < 0.001$.

FIG. 3. Effect of T3 on hCG secretion by normal and IUGR cytotrophoblasts. The hCG secretion at 48 h after T3 treatment (normal, $n = 9$; IUGR, $n = 6$) and 72 h after T3 treatment (normal, $n = 8$; IUGR, $n = 6$) was assessed and normalized to the hCG secretion at 0 h of T3 treatment (18 h after culture), which was given an arbitrary value of 100%. **, $P < 0.01$; ***, $P < 0.001$.

FIG. 4. T3 transport by normal and IUGR cytotrophoblasts. A, T3 uptake. Cytotrophoblasts were incubated with 1 nM T3 containing $2 \times 10^5$ cpm $^{125}$I-T3 for 5 to 30 min, and the amount of cellular radioactivity was assessed (normal, $n = 7$; IUGR, $n = 5$). B, T3 efflux. Cytotrophoblasts were incubated for 10 min with 1 nM T3 containing $2 \times 10^5$ cpm $^{125}$I-T3 and after brief washing were incubated with SFM without T3 (efflux media) for 0 to 10 min. The amount of radioactivity that was retained intracellularly was assessed and expressed as a percentage of the cellular radioactivity before the addition of efflux media (0 min) (normal, $n = 6$; IUGR, $n = 5$). ***, $P < 0.001$. 

MCT8 and MCT10 expression in normal and IUGR cytotrophoblasts

Changes in the expression of TH transporters may account for the altered T3 transport in IUGR cytotropho-
blasts. In previous studies, we have reported that the expression of MCT8 and MCT10 is altered in IUGR villous placental biopsies, which consist of multiple cell types (24, 25). We thus sought to investigate the mRNA and protein expression of MCT8 and MCT10 in primary cultures of IUGR and normal cytotrophoblasts after 18 h of culture (presyncytialization) or 66 h (postsyncytialization). There were no differences in the expression of MCT8 or MCT10 mRNA in IUGR compared with normal cytotrophoblasts at both 18 and 66 h after culture (Fig. 5, A and B). However, Western immunoblottings and their quantification by relative densitometry demonstrated that MCT8 protein expression was higher in IUGR compared with normal cytotrophoblasts (\( P < 0.01 \)) (Fig. 5C), most significantly at 18 h by 2.1-fold (\( P < 0.05 \)). In contrast, there was no difference in the protein expression of MCT10 between IUGR and normal cytotrophoblasts (Fig. 5D). In addition, overall the expression of MCT8 and MCT10 in cytotrophoblasts decreased significantly between 18 and 66 h after culture at both the mRNA (\( P < 0.001 \)) and protein (MCT8, \( P < 0.001 \); MCT10, \( P < 0.05 \)) levels. Relative quantification of the mRNA encoding the TH transporters OATP1A2, OATP4A1, LAT1, LAT2, and CD98, which have been reported in the human placenta, revealed no significant differences between IUGR and normal cytotrophoblasts (data not shown).

**Discussion**

In this study, we have demonstrated that whereas cytotrophoblasts isolated from normal placentae are unaffected by T3 treatment, cytotrophoblasts from IUGR placentae are responsive to T3 in terms of cell survival and apoptosis. This effect is associated with increased accumulation of intracellular T3 in IUGR cytotrophoblasts and increased protein expression of the TH transporter, MCT8. In this report, we show for the first time that the previously documented increase in apoptosis by IUGR placenta (11–15) may be partly mediated by T3.

In accordance with our previous study (26), we have confirmed that T3 does not affect the survival of normal cytotrophoblasts. In contrast, T3 adversely affects the survival of IUGR cytotrophoblasts. We also demonstrated that T3 induces apoptosis in IUGR, but not in normal cytotrophoblasts *in vitro*, indicating that the effect of T3 on the survival of IUGR cytotrophoblasts may be mediated via increased apoptosis. The regulation of apoptosis in the villous trophoblast is important for normal placental development. The syncytiotrophoblast layer of the placenta, which is the cell barrier that controls transplacental transport, is continuously renewed by cytotrophoblasts fusing into adjacent syncytiotrophoblast, whereas aged syncytiotrophoblast nuclei are extruded into the maternal circulation as...
“syncytial knots.” It is believed that this process is regulated by apoptotic mechanisms (33). IUGR has been associated with increased apoptosis in the human placenta, as demonstrated by more apoptotic nuclei seen histologically and increased caspase 3 activity in IUGR compared with normal placentae, particularly in syncytiotrophoblasts (11–13). It has been shown before that the IUGR placenta is more susceptible to apoptotic stimuli such as TNF-α and hypoxia (14, 34). However, this is the first study to show that T_3 can also induce apoptosis in IUGR cytotrophoblasts in vitro, whereas it has no effect on normal cytotrophoblasts.

Primary cultures of cytotrophoblasts isolated from term placentae syncytialize with time in culture to form multinucleate cells. This differentiation process is associated with increased secretion of hCG (28). Our finding of increased hCG secretion by IUGR compared with normal cytotrophoblasts is in agreement with other studies. Crocker et al. (14) have found that hCG secretion was higher from explants from IUGR placentae compared with normal controls. Newhouse et al. (15) reported that there was increased syncytialization of primary cytotrophoblast cultures from IUGR compared with normal placentae assessed by immunofluorescent staining for desmoplakin and by hCG secretion. A previous study by Nishii et al. (35) suggested that treatment with 10 nM T_3 promotes hCG secretion in cytotrophoblasts. In our study, however, treatment with 10 nM T_3 did not significantly affect hCG secretion by either normal or IUGR cytotrophoblasts.

TH transport may play a role in regulating the impact of T_3 on cytotrophoblasts via regulating intracellular T_3 availability. We observed in vitro that net T_3 transport is increased in cytotrophoblasts from IUGR compared with normal placentae at 66 h after culture, whereas T_3 efflux is unaltered, thus indicating that there is increased intracellular accumulation of T_3 in IUGR cytotrophoblasts, which may contribute to their increased sensitivity to T_3 treatment. The increased intracellular accumulation of T_3 within IUGR cytotrophoblasts may be due to changes in TH transporter expression, changes in T_3-binding capacity within cytotrophoblasts, as well as differences in individual cell volume due to the increased syncytialization of IUGR cytotrophoblasts.

We have previously reported that the protein expression of MCT8 is increased in whole placental biopsies from pregnancies complicated by severe IUGR requiring delivery in the early third trimester compared with gestationally matched controls, but not in placentae from IUGR pregnancies delivered after 37 wk (24, 25). In contrast, the present results obtained using primary cultures of cytotrophoblasts (>95% pure) revealed that MCT8 protein expression was also increased in cytotrophoblasts from late third trimester IUGR placentae. This change was not accompanied by an increase in MCT8 mRNA expression indicating that posttranscriptional or posttranslational modulation occurs. We have previously reported such discrepancies between mRNA and protein expression for MCT8 (25), and similar findings have been reported for other plasma membrane transporters (36).

Increased protein expression of MCT8 is likely to contribute to the increased net T_3 uptake observed in IUGR cytotrophoblasts. Although MCT8 can facilitate T_3 efflux (31), our findings suggest that T_3 uptake may be the more dominant role of MCT8 in cytotrophoblasts. Although we found no changes in the mRNA or protein expression of MCT10 or in the mRNA expression of LATs or OATPs in IUGR cytotrophoblasts, consistent with previous findings in whole placental biopsies (25), we cannot exclude the possibility that changes in the activity of these TH transporters may also contribute to the increased net T_3 uptake by IUGR cytotrophoblasts. In addition, increased accumulation of T_3 within IUGR cytotrophoblasts may occur as a result of the possible increased intracellular binding of T_3 to TR isoforms, TRα1 and TRβ1, that have previously been found to be expressed at increased levels in IUGR placenta villi (22). If so, this could contribute to both increased intracellular accumulation of T_3 and increased sensitivity of IUGR cytotrophoblasts to T_3.

Unlike findings in central nervous system cell types (37), treatment of term cytotrophoblasts with T_3 concentrations within the physiological range (10 nm or less) does not alter the expression of the deiodinase enzymes, D2 (activates T_4 to T_3) and D3 (inactivates T_3) (23). Furthermore, no significant changes were observed in D2 and D3 mRNA expression and activity in biopsies from normal compared with IUGR placentae (23). This suggests that cytotrophoblasts cannot modulate intracellular T_3 concentration through alterations in D2 and D3 activities, thus rendering IUGR cytotrophoblasts more vulnerable to the increased net T_3 uptake demonstrated in this current study.

The increased T_3 responsiveness of IUGR cytotrophoblasts could be a contributing factor to the underlying pathogenesis of IUGR or a consequence of this malplacentation syndrome. Maternal hyperthyroidism is one of the endocrine factors that have been associated with IUGR (16), suggesting that increased exposure to TH may be detrimental to fetoplacental development. Outside the context of maternal thyroid disorders, our findings that there is increased T_3 accumulation within IUGR cytotrophoblasts and that T_3 can adversely affect the survival and increase apoptosis of these cells raise the possibility that an abnormally high T_3 concentration locally forms part of the...
dysregulated endocrine, paracrine, and autocrine environment, which occurs within the placenta in IUGR pregnancies. Further investigation into the possible etiologies and pathophysiology of altered T3 responsiveness within placental trophoblasts in IUGR is warranted.

Acknowledgments

We thank the United Kingdom Clinical Research Network-funded research midwives at Birmingham Women’s Hospital for assistance with patient recruitment and Dr. Roger Holder (University of Birmingham) for statistical advice.

Address all correspondence and requests for reprints to: Dr. Shiao Chan, School of Clinical and Experimental Medicine, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom. E-mail: s.chan@bham.ac.uk.

This work was supported the Medical Research Council (Grants G0501548, to S.-Y.C., M.D.K., J.A.F., and C.J.M.; and G0600285, to S.-Y.C. and M.D.K.), Action Medical Research (Grant SP4335, to M.D.K., S.-Y.C., L.S.L., and J.A.F.), and Wellbeing of Women (Grant RG/1082/09, to S.-Y.C., M.D.K., J.A.F., and L.S.L.). S.-Y.C. is supported by a Clinician Scientist Fellowship (6462/4335) awarded by the Health Foundation.

Disclosure Summary: The authors have nothing to disclose.

References

21. Yen PM 2001 Physiological and molecular basis of thyroid hormone action. Physiol Rev 81:1097–1142


Main Document:

1. Definitions

a. "Collective Work" means a work, such as a periodical issue, anthology or encyclopedia, in which the Work in its entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole. A work that constitutes a Collective Work will not be considered a Derivative Work for the purposes of this License.

b. "Derivative Work" means a work based upon the Work or upon the Work and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Work may be recast, transformed, or adapted, except that a work that constitutes a Collective Work will not be considered a Derivative Work for the purpose of this License.

c. "Licensee" means the individual or entity that offers the Work under the terms of this License.

d. "Original Author" means the individual or entity who created the Work.

e. "Work" means the copyrightable work of authorship offered under the terms of this License.

f. "You" means an individual or entity exercising rights under this License who has not previously violated the terms of this License with respect to the Work, or who has received express permission from the Licensor to exercise rights under this License despite a previous violation.

2. Fair Use Rights. Nothing in this license is intended to reduce, limit, or restrict any rights arising from fair use, first sale or other limitations on the exclusive rights of the copyright owner under copyright law or other applicable laws.

3. License Grant. Subject to the terms and conditions of this License, Licensor hereby grants You a worldwide, royalty-free, non-exclusive, perpetual (for the duration of the applicable copyright) license to exercise the rights in the Work as stated below:

   a. to reproduce the Work, to incorporate the Work into one or more Collective Works, and to reproduce the Work as incorporated in the Collective Works;
   
   b. to create and reproduce Derivative Works;
   
   c. to distribute copies or phonorecords of, display publicly, perform publicly, and perform publicly by means of a digital audio transmission the Work including as a Derivative Work, and Derivative Works;
   
   d. to distribute copies or phonorecords of, display publicly, perform publicly, and perform publicly by means of a digital audio transmission Derivative Works;

   The above rights may be exercised in all media and formats whether now known or hereafter devised. The above rights include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. All rights not expressly granted by Licensor are hereby reserved, including but not limited to the rights set forth in Sections 4(d) and 4(e).

4. Restrictions.

The license granted in Section 3 above is expressly made subject to and limited by the following restrictions:

   a. You may distribute, publicly display, publicly perform, or publicly digitally perform the Work only under the terms of this License, and You must include a copy of, or the Uniform Resource Identifier for, this License with every copy or phonorecord of the Work You distribute, publicly display, publicly perform, or publicly digitally perform. You may not offer or impose any terms on the Work that alter or restrict the terms of this License or the recipients' exercise of the rights granted hereunder. You may not sublicense the Work. You must keep intact all notices that refer to this License and to the disclaimer of warranties. You may not distribute, publicly display, publicly perform, or publicly digitally perform the Work with any technological measures that control access or use of the Work in a manner inconsistent with the terms of this License Agreement. The above applies to the Work as incorporated in a Collective Work, but this does not require the Collective Work apart from the Work itself to be made subject to the terms of this License. If You create a Collective Work, upon notice from any Licensor You must, to the...
extent practicable, remove from the Collective Work any reference to such Licensor or the Original Author, as requested. If you create a Derivative Work, upon notice from any Licensor You must, to the extent practicable, remove from the Derivative Work any reference to such Licensor or the Original Author, as requested.

b. You may not exercise any of the rights granted to You in Section 3 above in any manner that is primarily intended for or directed toward commercial advantage or private monetary compensation. The exchange of the Work for other copyrighted works by means of digital file-sharing or otherwise shall not be considered to be intended for or directed toward commercial advantage or private monetary compensation, provided there is no payment of any monetary compensation in connection with the exchange of copyrighted works.

c. If you distribute, publicly display, publicly perform, or publicly digitally perform the Work or any Derivative Works or Collective Works, You must keep intact all copyright notices for the Work and give the Original Author credit reasonable to the medium or means You are utilizing by conveying the name (or pseudonym if applicable) of the Original Author if supplied; the title of the Work if supplied; to the extent reasonably practicable, the Uniform Resource Identifier, if any, that Licensor specifies to be associated with the Work, unless such URI does not refer to the copyright notice or licensing information for the Work; and in the case of a Derivative Work, a credit identifying the use of the Work in the Derivative Work (e.g., “French translation of the Work by Original Author,” or “Screenplay based on original Work by Original Author”). Such credit may be implemented in any reasonable manner; provided, however, that in the case of a Derivative Work or Collective Work, at a minimum such credit will appear where any other comparable authorship credit appears and in a manner at least as prominent as such other comparable authorship credit.

d. For the avoidance of doubt, where the Work is a musical composition:

i. Performance Royalties Under Blanket Licenses. Licensor reserves the exclusive right to collect, whether individually or via a performance rights society (e.g. ASCAP, BMI, SESAC), royalties for the public performance or public digital performance (e.g. webcast) of the Work if that performance is primarily intended for or directed toward commercial advantage or private monetary compensation.

ii. Mechanical Rights and Statutory Royalties. Licensor reserves the exclusive right to collect, whether individually or via a music rights agency or designated agent (e.g. Harry Fox Agency), royalties for any phonorecord You create from the Work (“cover version”) and distribute, subject to the compulsory license created by 17 USC Section 115 of the US Copyright Act (or the equivalent in other jurisdictions), if Your distribution of such cover version is primarily intended for or directed toward commercial advantage or private monetary compensation.

iii. Webcasting Rights and Statutory Royalties. For the avoidance of doubt, where the Work is a sound recording, Licensor reserves the exclusive right to collect, whether individually or via a performance-rights society (e.g. SoundExchange), royalties for the public digital performance (e.g. webcast) of the Work, subject to the compulsory license created by 17 USC Section 114 of the US Copyright Act (or the equivalent in other jurisdictions), if Your public digital performance is primarily intended for or directed toward commercial advantage or private monetary compensation.

5. Representations, Warranties and Disclaimer

UNLESS OTHERWISE MUTUALLY AGREED TO BY THE PARTIES IN WRITING, LICENSOR OFFERS THE WORK AS IS AND MAKES NO REPRESENTATIONS OR WARRANTIES OF ANY KIND CONCERNING THE WORK, EXPRESS, IMPLIED, STATUTORY OR OTHERWISE, INCLUDING, WITHOUT LIMITATION, WARRANTIES OF TITLE, MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, NONINFRINGEMENT, OR THE ABSENCE OF LATENT OR OTHER DEFECTS, ACCURACY, OR THE PRESENCE OF ABSENCE OF ERRORS, WHETHER OR NOT DISCOVERABLE. SOME JURISDICTIONS DO NOT ALLOW THE EXCLUSION OF IMPLIED WARRANTIES, SO SUCH EXCLUSION MAY NOT APPLY TO YOU.

6. Limitation on Liability. EXCEPT TO THE EXTENT REQUIRED BY APPLICABLE LAW, IN NO EVENT WILL LICENSOR BE LIABLE TO YOU ON ANY LEGAL THEORY FOR ANY SPECIAL, INCIDENTAL, CONSEQUENTIAL, PUNITIVE OR EXEMPLARY DAMAGES ARISING OUT OF THIS LICENSE OR THE USE OF THE WORK, EVEN IF LICENSOR HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES.

7. Termination

a. This License and the rights granted hereunder will terminate automatically upon any breach by You of the terms of this License. Individuals or entities who have received Derivative Works or Collective Works from You under this License, however, will not have their licenses terminated provided such individuals or entities remain in full compliance with those licenses. Sections 1, 2, 5, 6, 7, and 8 will survive any termination of this License.

b. Subject to the above terms and conditions, the license granted here is perpetual (for the duration of the applicable copyright in the Work). Notwithstanding the above, Licensor reserves the right to release the Work under different license terms or to stop distributing the Work at any time; provided, however, that any such election will not serve to withdraw this License (or any other license that has been, or is required to be, granted under the terms of this License), and this License will continue in full force and effect unless terminated as stated above.

8. Miscellaneous

a. Each time You distribute or publicly digitally perform the Work or a Collective Work, the Licensor offers to the recipient a license to the Work on the same terms and conditions as the license granted to You under this License.

b. Each time You distribute or publicly digitally perform a Derivative Work, Licensor offers to the recipient a license to the original Work on the same terms and conditions as the license granted to You under this License.

c. If any provision of this License is invalid or unenforceable under applicable law, it shall not affect the validity or enforceability of the remainder of the terms of this License, and without further action by the parties to this agreement, such provision shall be reformed to the minimum extent necessary to make such
d. No term or provision of this License shall be deemed waived and no breach consented to unless such waiver or consent shall be in writing and signed by the party to be charged with such waiver or consent.

e. This License constitutes the entire agreement between the parties with respect to the Work licensed here. There are no understandings, agreements or representations with respect to the Work not specified here. Licensor shall not be bound by any additional provisions that may appear in any communication from You. This License may not be modified without the mutual written agreement of the Licensor and You.

Creative Commons is not a party to this License, and makes no warranty whatsoever in connection with the Work. Creative Commons will not be liable to You or any party on any legal theory for any damages whatsoever, including without limitation any general, special, incidental or consequential damages arising in connection to this license. Notwithstanding the foregoing two (2) sentences, if Creative Commons has expressly identified itself as the Licensor hereunder, it shall have all rights and obligations of Licensor.

Except for the limited purpose of indicating to the public that the Work is licensed under the CCPL, neither party will use the trademark “Creative Commons” or any related trademark or logo of Creative Commons without the prior written consent of Creative Commons. Any permitted use will be in compliance with Creative Commons’ then-current trademark usage guidelines, as may be published on its website or otherwise made available upon request from time to time.

Creative Commons may be contacted at http://creativecommons.org/.