0021-972X/96/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 81, No. 6
Printed in U.S.A.

Type II and Type III Deiodinase Activity in Human Placenta as a Function of Gestational Age

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ABSTRACT

Thyroid hormones are essential for fetal development. T_4 can be activated by type I (ID-I) and type II (ID-II) iodothyronine deiodinase or inactivated by type III deiodinase (ID-III). The influence of placental ID-II and ID-III on the regulation of fetal thyroid hormone levels was investigated. Using [^{125}I]T_4 and [^{125}I]T_3, respectively, ID-II and ID-III activities were measured in homogenates of normal human placentas from 6–43 weeks gestational age and in placentas from five term neonates with a total thyroid hormone synthesis defect.

ID-II and ID-III activities related to protein or DNA concentration decreased and total placental ID-III activity increased significantly during pregnancy, whereas the increase in total placental ID-II activity was not significant.

Absolute placental ID-II activity was approximately 200 times lower than ID-III activity at all gestational ages. Therefore, fluctuations in ID-II activity were not likely to have a significant influence on fetal thyroid hormone concentrations, but may play a role in the regulation of intraplacental T_3 generation. The high ID-III activity most likely influences the thyroid hormone economy of the fetus. Severely hypothyroid newborns showed strongly decreased serum T_4 levels, but serum T_3 and placental ID-III activities were similar to those in euthyroid newborns. These results suggest that placental ID-III activity is regulated by serum T_3, but not by serum T_4. (*J Clin Endocrinol Metab* **81:** 2154–2158, 1996)

IT IS GENERALLY accepted that thyroid hormones are essential in all phases of brain development (see Ref. 1 for review). The human fetal thyroid only begins to produce substantial amounts of T_4 at midgestation (2, 3). Accordingly, early fetal development is dependent on the maternal T_4 supply. Evidence for T_4 passage across the placenta was obtained from studies in which T_4 could be detected in human fetuses before the onset of fetal thyroid function (4). Also, at term thyroid hormones from maternal origin can cross the placenta and reach the fetus without being deiodinated, as was shown by Vulsma *et al.* (5), who measured 35–70 nmol T_4/L (normal, 100–130 nmol/L) in cord blood serum from human term neonates who were unable to produce any thyroid hormone. Fetal serum T_4 and T_3 levels may be regulated by various mechanisms, *i.e.* the hypothalamic-pituitary-thyroid negative feedback system, the T_4 supply from the mother, and/or T_4 (in)activation. These last processes are catalyzed by iodothyronine deiodinases converting T_4 to the active metabolite T_3 or the inactive metabolite rT_3. Three types of deiodinases have been described. They differ in the mechanism of deiodination, localization, substrate preference, and 6-propylthiouracil (PTU) sensitivity (see Refs. 6 and 7 for review). Type I deiodinase (ID-I) catalyzes the inner and outer ring deiodination, is abundant in liver and kidney, prefers rT_3 as substrate, and is inhibited by PTU. Type II deiodinase (ID-II) is an outer ring deiodinase found primarily in brain, pituitary, and brown adipose tissue and also in placenta; has a preference for T_4 as substrate; and is PTU insensitive. Finally, type III deiodinase (ID-III) catalyzes the inner ring deiodination of T_4 and T_3, is mainly present in placenta and brain, prefers T_3 as substrate, and is also PTU insensitive. ID-III is considered to inactivate thyroid hormone, whereas ID-II activates thyroid hormone, and ID-I is capable of both activation and inactivation. ID-II and ID-III are regulated by thyroid hormone concentrations. In response to hypothyroidism, ID-III activity in brain decreases (8–10), whereas ID-II activity increases (8, 11, 12). The reverse occurs in hyperthyroidism. Because in rat placenta, T_3 and T_4 concentrations increase during pregnancy (13), and ID-II and ID-III activities are present in placenta, these enzymes could have an important function in regulating fetal thyroid hormone levels.

To study this hypothesis we measured ID-II and ID-III activities in 69 human placentas from 6–43 weeks gestation.

Materials and Methods

Placental tissues

Sixty-nine placentas, evenly distributed over 6–43 weeks gestational age, were collected immediately after delivery. Also, five placentas were obtained from hypothyroid newborns. This study was approved by the committee of medical ethics of our hospital. Villous structures of placentas were isolated, frozen in liquid nitrogen, and stored at −80 C, usually within 2 h. Placenta sections (1 g) were homogenized in 4 mL 10 mmol HEPES/L (pH 7.2), 250 mmol sucrose/L, and 10 mmol dithiothreitol (DTT)/L. Homogenates were stored at −80 C until further use.

Received October 11, 1995. Revision received January 3, 1996. Accepted January 11, 1996.

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ID-II activity assay

Just before use, [^{125}I]T_4 was purified on 1-mL bed volume Sephadex LH-20 columns. Columns were successively eluted with 0.1 mol HCl/L (3 × 1 mL) to remove iodide, with H_2O (3 × 1 mL), and with 1% ammonia in ethanol (1 × 750 μL, 1 × 1.2 mL, and 1 × 1 mL). The 1.2-mL fraction contained purified [^{125}I]T_4 and was used for the incubations. ID-II activity was determined by incubating placental homogenates (2.5 and 5 mg protein/mL) for 90 min at 37 C with approximately 100,000 cpm [^{125}I]T_4 (~0.3 nmol/L) and 1 nmol nonradioactive T_4/L in 0.2 mol sodium phosphate/L (pH 7.2), 4 mmol sodium ethylenediamine tetraacetate/L, and 20 mmol DTT/L, in a final volume of 200 μL. The total protein concentration in all assay mixtures was set at 5 mg/mL by the addition of BSA. To prevent inner ring deiodination of tracer T_4 and newly produced labeled T_3, 100 nmol T_3/L were added to the incubation mixture. The reaction was stopped by the addition of 100 μL 4.5% (wt/vol) BSA, immediately followed by 500 μL 10% (wt/vol) TCA. After centrifugation, the supernatant, containing acid-soluble radioiodine, was separated from the precipitate, containing iodothyronines, and both fractions were counted in a γ-counter.

To determine the K_m of ID-II for T_4, 0, 1.5, 2.5, and 3.5 nmol nonradioactive T_4/L were added to the homogenate that already contained endogenous T_4.

ID-II activity was expressed as femtomoles of T_4 converted per min/mg protein or per μg DNA, and the maximum velocity (V_{max}) was calculated using the formula: $V_{max} = v(K_m/[S] + 1)$.

Product formation was corrected for nonenzymatic deiodination observed in incubations with 1 μmol nonradioactive T_4/L. This control value always amounted to approximately 8% of the radioactivity added, which was the same when 2.5 or 5 mg BSA/mL was used. A factor of 2 was applied to the corrected value, because [^{125}I]T_4 is randomly labeled in the 3′- and 5′-positions. ID-II activity in homogenates was linear with incubation time until at least 120 min and with protein concentrations up to 5 mg/mL. All reactions were performed in duplicate in three separate experiments.

ID-III activity assay

ID-III activity was determined as described previously (14). Briefly, placental homogenates (0.14–0.43 mg protein/mL) were incubated for 30 min at 37 C with approximately 100,000 cpm [^{125}I]T_3 (~0.6 nmol/L) and 1 nmol/L nonradioactive T_3 in 0.2 mol sodium phosphate/L (pH 7.2), 4 mmol sodium ethylenediamine tetraacetate/L, and 25 mmol DTT/L in a total volume of 35 μL. After incubation, the reaction was stopped by the addition of 50 μL methanol. T_3, 3,3′-diiodothyronine (3,3′-T_2), and iodide were separated by silica gel thin layer chromatography and quantified by phosphor imaging (Molecular Dynamics, Sunnyvale, CA). The radioactivity in the T_3 and 3,3′-T_2 spots was calculated as the percentage of total radioactivity in the two spots.

ID-III activity was expressed as femtomoles of T_3 converted per min/mg protein or per μg DNA, and the V_{max} of ID-III was calculated, using a K_m of 3.4 nmol/L (14).

Product formation was corrected for nonenzymatic 5-deiodination observed in the incubations without placental homogenates. No decrease in ID-III activity was observed when placentas were left at room temperature for up to 3 h before preparation of the homogenate. Care was taken that 3,3′-T_2 was not further deiodinated to T_1. The addition of 1 μmol nonradioactive T_3/L completely blocked [^{125}I]T_2 formation. ID-III activity in homogenates was linear with incubation time until at least 60 min and with protein concentrations up to 1 mg/mL. All reactions were performed in duplicate in three separate experiments.

Other assays

Protein concentrations were determined using the method of Bradford (15), with BSA as standard.

DNA concentrations were measured with the method of Burton (16) modified by Gendimenico *et al.* (17).

Endogenous T_4 levels in placental tissue were determined as follows: 1 g placental tissue was homogenized in 4 mL alkaline methanol, with or without 10,000 cpm [^{125}I]T_4 added to calculate recovery, and the homogenate was incubated while shaking for 1 h at room temperature to dissolve T_4. After centrifugation, the supernatant was incubated with Bio-Rad AG1x2 (200–400 mesh, in chloride form) for 1 h at room temperature to bind T_4 and eluted according to the method of Mallol *et al.* (18). The fractions containing T_4 were evaporated under nitrogen and resuspended in 200 μL RIA buffer, and the T_4 concentration was measured by RIA (19). Taking into account a recovery of approximately 50%, 10, 50, and 100 nmol/L T_4 added to a placental homogenate gave correct concentrations in the RIA.

Fetal hypothyroidism was confirmed by measuring T_3, T_4, and TSH in cord serum by RIA (19).

Statistical analysis

To discern a trend, Spearman's r was calculated.

Results

ID-II activity assay

A considerable amount of blood is present in placenta (20, 21). As both blood and placental tissue contain T_4, we measured endogenous T_4 concentrations in four placental homogenates of 15, 20, 38, and 41 weeks gestation. Endogenous T_4 might interfere with ID-II measurement, resulting in the underestimation of its activity. Endogenous T_4 levels in the ID-II assay mixture containing placental homogenates were approximately 1.5 nmol/L in the four placentas, which is not negligible compared to the approximately 1.3 nmol/L added substrate concentration. To correct for the T_4 contribution of the placental homogenates, the K_m was determined. The K_m of ID-II for T_4 was approximately 6 nmol/L (Fig. 1). Representing a value independent of the endogenous T_4 concentration, the V_{max} of ID-II was calculated for each sample using this K_m value and the total T_4 concentration.

ID-III activity assay

The endogenous T_3 concentration in the ID-III assay mixture containing placental homogenates was 3.31 ± 1.32 pmol/L, whereas 1.7 nmol/L was added as substrate. For that reason, the contribution of endogenous T_3 could be neglected.

ID-II and ID-III activities during pregnancy

Figure 2 shows that both ID-II and ID-III activities per mg protein decreased during pregnancy ($r_s = -0.41$; $P = 0.001$ and $r_s = -0.67$; $P < 0.0001$, respectively), with ID-II activity

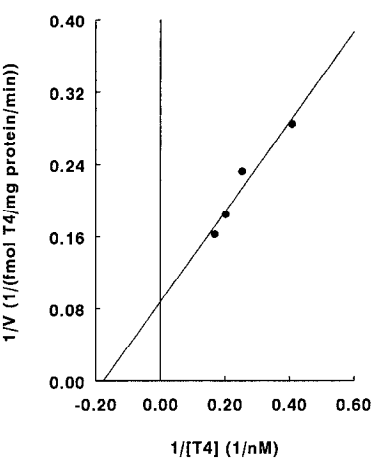

FIG. 1. Double reciprocal plot of ID-II activity as a function of the T_4 concentration in human placental homogenates. The K_m is 6 nmol/L. The V_{max} is 11.1 fmol T_4 converted/min·mg protein.

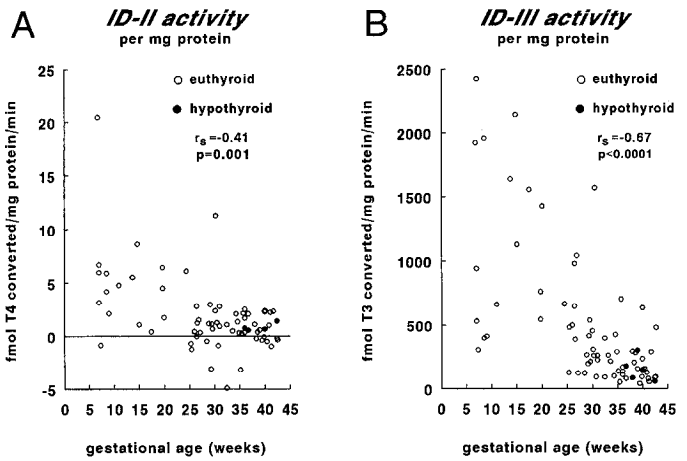

FIG. 2. ID-II and ID-III activities in human placental homogenates expressed per mg protein in relation to gestational age. ○ Euthyroid fetuses; ●, hypothyroid fetuses. Results are from one representative experiment, performed in duplicate. Three separate experiments were conducted.

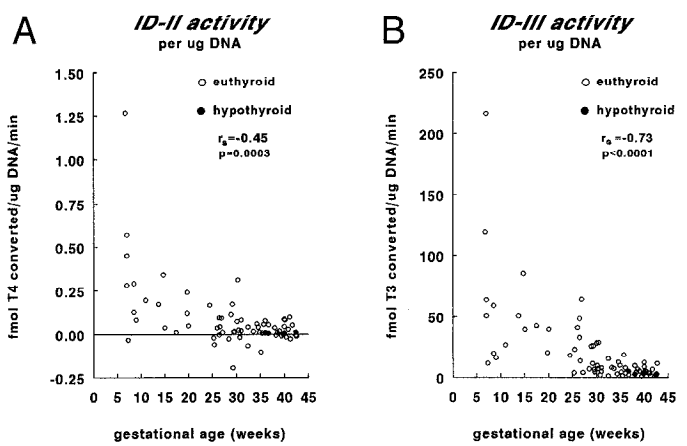

FIG. 3. ID-II and ID-III activities in human placental homogenates from euthyroid fetuses expressed per μg DNA in relation to gestational age. ○, Euthyroid fetuses; ●, hypothyroid fetuses. Results are from one representative experiment, performed in duplicate. Three separate experiments were conducted.

declining to virtually zero at term. However, protein concentration increased with gestational age (r_s = 0.67; $P <$ 0.0001). As variable amounts of blood proteins can contribute considerably to the protein concentration of the placenta, ID-II and ID-III activities were also expressed per μg DNA. ID-II and ID-III activities relative to DNA also decreased during pregnancy (Fig. 3; $r_s = -0.45$; $P = 0.0003$ and $r_s = -0.73$; $P < 0.0001$, respectively). Also, DNA concentration increased with gestational age (r_s = 0.72; $P < 0.0001$).

ID-II activity relative to protein or DNA was approximately 200 times lower than ID-III activity at all gestational ages.

When total placental ID-III activity was calculated, a progressive increase with gestational age was observed (Fig. 4; r_s = 0.45; P = 0.0003).

Effect of fetal hypothyroidism on ID-II and ID-III activities

T_4, T_3, and TSH concentrations in cord blood from five hypothyroid neonates are shown in Table 1. All five children have

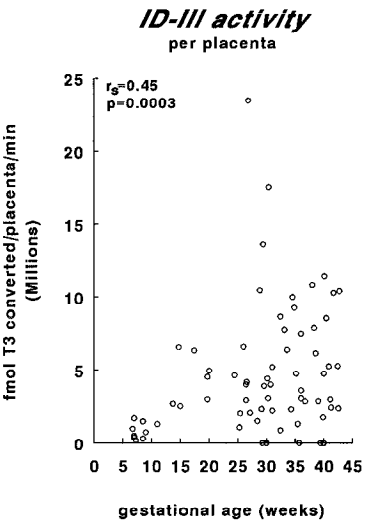

FIG. 4. Total placental ID-III activity in human placentas from euthyroid fetuses in relation to gestational age.

a total iodide organification defect due to thyroid peroxidase gene mutations (22) and are unable to produce any thyroid hormone. T_4 levels were considerably lower than those in cord blood plasma from euthyroid neonates, whereas TSH concentrations were much higher (5). These data indicate severe hypothyroidism. On the contrary, T_3 concentrations were not different from those in euthyroid neonates.

To study the effect of fetal hypothyroidism on ID-II and ID-III activities, the placentas were analyzed for ID-II and ID-III activities and compared with those in placentas from euthyroid neonates.

As in placentas from term euthyroid neonates, ID-II activity per mg protein or per μg DNA was relatively low in the five term placentas from hypothyroid neonates compared to the ID-III activity (Table 1 and Figs. 2a and 3a).

ID-III activity relative to protein or DNA of placentas from hypothyroid neonates was in the same range as ID-III activity in placentas of euthyroid neonates (Table 1 and Figs. 2b and 3b). Also, total placental ID-III activity in placentas from hypothyroid neonates was not significantly different from that in euthyroid neonates (results not shown).

Discussion

In this study ID-II and ID-III activities in human placentas during the entire gestational period were measured. As thyroid hormone (*i.e.* T_4) is activated by ID-II or inactivated by ID-III, the relative activities of these enzymes in placenta may regulate the amount of active thyroid hormone available for the fetus.

Role of ID-II in placenta

Until now, controversy has existed about the presence of ID-II activity in term human placentas. Kaplan *et al.* (23) detected ID-II activity mainly in mixed fetal membranes, but also in trophoblasts, whereas others could not detect ID-II activity in the placenta (24, 25). In this study we show that ID-II activity relative to protein or DNA concentration declined during pregnancy, from 8 fmol T_4 converted/min·mg protein, or 0.5 fmol/μg DNA/min in the first trimester, to

TABLE 1. T_4, T_3, and TSH concentrations in cord blood plasma, and ID-II and ID-III activities relative to protein and DNA in placental homogenates from 5 hypothyroid term neonates and 18 euthyroid term newborns (mean ± SD)

	T_3 (nmol/L)	T_4 (nmol/L)	TSH (mU/L)	ID-II (fmol T_4/mg protein · min)	ID-II (fmol T_4/ μg DNA · min)	ID-III (fmol T_3/mg protein · min)	ID-III (fmol T_3/μg DNA · min)
Hypothyroid							
1	1.1	40	147	ND	ND	303	ND
2	1.0	45	490	1.53	0.041	60	1.61
3	0.6	55	1030	0.56	0.015	179	4.89
4	0.6	55	148	0.75	0.022	87	2.56
5	0.65	50	375	0.62	0.016	142	3.73
Mean ± SD	0.79 ± 0.24	49 ± 7	438 ± 363	0.87 ± 0.45	0.024 ± 0.012	154 ± 95	3.20 ± 1.42
Euthyroid							
Mean ± SD	0.77 ± 0.28[a]	163 ± 44[a]		1.04 ± 1.06	0.011 ± 0.013	198 ± 157	5.31 ± 3.37
Range			1–20[a]				

ND, Not determined.
[a] From Ref. 45.

hardly detectable activities at term. ID-II activity was about 200-fold lower than ID-III activity at all gestational ages, suggesting that placental ID-II activity has no significant influence on fetal thyroid hormone plasma concentrations, but may play a role in the regulation of local cellular T_3 concentration. This is also suggested by Burrow *et al.* (26) and is supposed to be the case for other tissues, such as brain and brown adipose tissue (27). The relatively high placental ID-II activity in the first trimester of pregnancy may generate sufficient local T_3 to induce differentiation of trophoblasts. Maruo *et al.* (28) have shown that endocrine functions, like progesterone, estradiol, hCG, and human placental lactogen secretion by trophoblasts in culture were optimally stimulated by T_3 (10 nmol/L) or T_4 (100 nmol/L). This was only observed in cultured early placental tissue. At the end of pregnancy, less T_3 will be formed locally in the placenta, but T_3 has lost the above-mentioned effects on cultured term placental tissues (28). In agreement with these observations, Ashitaka *et al.* (29) and Nishii *et al.* (30) showed that the binding capacity of nuclear T_3 receptors in human early placental trophoblasts was much higher than that in term placental cells.

Role of ID-III in placenta

It has been suggested that placental ID-III functions as a barrier for maternal T_4 and T_3 (24). In this study ID-III activity, expressed per mg protein or per μg DNA, decreased during gestation. This is in accordance with Yoshida *et al.* (31) in human placenta and shows the same trend as in rabbit placenta (32). Thus, in the first trimester, when the placenta and the transport surface area are small, there is high specific ID-III activity. At term, specific ID-III activity is decreased, but because the placenta and the surface area are much larger than those in a first semester placenta, the ID-III activity per placenta is increased. This increase in total ID-III activity per placenta could result in an overall increase in T_4 inactivation.

It seems rational to consider fetal thyroid hormone as one pool, regardless of whether it comes from the mother or the fetus. Regulation is not only at the level of T_4 transfer across the placenta, but fetal T_4 and T_3, produced by the fetal thyroid or derived from the mother, can be inactivated by ID-III or activated by ID-II in placenta, fetal brain, and various other fetal tissues.

In contrast to the increasing total placental ID-III activity during pregnancy, ID-III activity per mg protein or per μg DNA is decreasing as mentioned previously. This depends on the even stronger increase in protein and DNA during pregnancy, as shown in this study and by others (33–36). The increase in protein concentration is due to the fact that with rising gestational age, the placenta contains more connective tissue (33). Between 22–40 weeks of pregnancy, blood represents 20–30% of placental weight (20, 21, 34, 37). However, hardly any DNA is present in blood, as 90% of fetal red blood cells is nonnucleated as early as the tenth week of pregnancy, and at term, fetal and maternal blood cells consist of 95% and 96% erythrocytes, respectively (38, 39). Thus, the total DNA concentration mainly represents DNA from placental tissue and not from blood and, therefore, is a more reliable parameter to relate to ID-III activity than is the protein concentration.

In contrast to human placenta, specific ID-III activity in rat placenta increased 2-fold from day 14 until day 16 or 17 of pregnancy (40, 41). Thereafter, a decrease was observed, ascribed by the researchers to aging of the placenta. However, contrary to human placenta, protein and DNA concentrations per g tissue in rat placenta decreased during pregnancy from 12–19 days (1.6- and 2-fold, respectively) (42), which could partly explain the change in ID-III activity during rat pregnancy.

Effect of fetal hypothyroidism on ID-III activity

Vulsma *et al.* (5) measured 35–70 nmol T_4/L in cord blood plasma of term neonates who were unable to produce any thyroid hormone (normal, 100–130 nmol T_4/L). Consequently, this T_4 must be of maternal origin, most likely from transplacental transfer. In hypothyroid neonates, specific ID-III activity in placenta is similar to the activity in placentas of euthyroid neonates. It is remarkable that the T_3 concentration in cord serum from hypothyroid fetuses is comparable to that in euthyroid fetuses, in contrast to the low serum T_4 level. Thus, in placenta, ID-III activity is probably regulated by circulating T_3, and not by T_4. In addition to fetal hypothyroidism, maternal hypo- or hyperthyroidism has no effect on ID-III activity in the placenta (31, 43), also indicating that maternal T_4 does not regulate placental ID-III activity.

In conclusion, placental ID-II activity is extremely low compared to ID-III activity at all gestational ages. Therefore,

fluctuations in ID-II activity are not likely to have a significant influence on fetal thyroid hormone concentrations, but may play a role in the regulation of intraplacental T_3 generation and specific placental functions. However, at term, virtually no ID-II activity has been found.

The high ID-III activity most likely influences thyroid hormone economy of the fetus, especially by regulating fetal serum T_4 and T_3 concentrations. The exact physiological role of placental ID-III is still controversial. It has been suggested that the function of placental ID-III is to provide iodide to the fetus, independent of maternal iodine uptake (44). Thus, the total placental ID-III is responsible for increasing amounts of iodide during pregnancy, that will, through the circulation, be available to both the fetal and maternal thyroid glands and can be used by the fetus for the increasing production of thyroid hormones by the developing thyroid. Specific ID-III activity decreases during pregnancy, but total placental ID-III activity increases, implicating a higher turnover of thyroid hormones. Although serum T_4 is decreased, severely hypothyroid fetuses have neither significantly lower T_3 concentrations in cord serum nor decreases in placental ID-III activity. These data indicate that serum T_4 concentrations do not influence placental ID-III activity. Fetal serum T_3 concentrations produced from T_4 by ID-II and at the end of pregnancy by ID-I, or T_3 originating from the mother may have such a regulatory role.

Acknowledgment

We are grateful to Dr. J. Oosting for advice on statistics. We are also indebted to MR'70, Dr. J.-J.J.H.M. Erwich, and the Department of Obstetrics and Gynecology for kindly supplying placentas. We also acknowledge the Dr Ludgardine Bouwman foundation for support.

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