Chronic levothyroxine and acute T₃ treatments enhance the amplitude and time course of uterine contractions in human

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¹Department of Obstetrics and Gynecology, Université de Sherbrooke, Sherbrooke, Quebec, Canada; ²Department of Physiology and Biophysics, Université de Sherbrooke, Sherbrooke, Quebec, Canada; and ³Service of Endocrinology, Faculty of Medicine and health sciences, Université de Sherbrooke, Sherbrooke, Quebec, Canada

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Corriveau S, Pasquier JC, Blouin S, Bellabarba D, Rousseau É. Chronic levothyroxine and acute T₃ treatments enhance the amplitude and time course of uterine contractions in human. Am J Physiol Endocrinol Metab 304: E478–E485, 2013. First published December 18, 2012; doi:10.1152/ajpendo.00346.2012.—This study compares the functional consequences of levothyroxine (T₄) treatment during pregnancy as well as the acute affects of triiodothyronine (T₃) on spontaneous uterine contractile activities observed in vitro. Uterine biopsies were obtained from consenting women undergoing elective caesarean at term (n = 28). Spontaneous contractile activities from T₄-treated pregnant women (n = 8) were compared with control patients (n = 20) by isometric tension measurements. Effects of acute T₃ and T₄ on control tissues were also monitored. Area under the curve, amplitude, time to peak, duration, and frequency were quantified. In uterine strips from women treated for hypothyroidism, phasic uterine contractions of larger amplitude (+77%) were observed, with a prolonged duration at 90% relaxation (+138%) and reduced frequency (−55%) compared with values of the control group. The addition of exogenous T₃ in vitro on control strips induced a significant increase in the duration of the contractions and a significant decrease in frequency (P < 0.05), which partially mimics the results obtained in strips from T₄-treated women. Significant modifications of contractile properties were observed in strips from pregnant women treated with levothyroxine, consistent with those observed with the addition of exogenous T₃. Clinical practices of modern obstetrics should take into account the effect of thyroid hormones on uterine contractions’ time course to ensure a tighter followup at the end of pregnancy to achieve safer delivery.

IN CANADA, APPROXIMATELY 10–20% OF WOMEN are affected by thyroid dysfunction, and prevalence of hypothyroidism can reach up to 4% in women of reproductive age (4, 5). In women, fertility and reproduction can be compromised by a clinical or subclinical hypothyroidism. Pregnancy is a factor that can modify the course of thyroid disease. Moreover, routine thyroid screening, diagnosis, and management of subclinical thyroid disorders in pregnancy are currently the subject of debate (12). However, women who suffer from thyroid dysfunction often display infertility and impaired obstetric outcomes during pregnancy (33). Indeed, an impaired obstetric outcome during pregnancy increases the risk of complications, notably miscarriages, preeclampsia, incidence of still births, placental abruption, and premature deliveries (1, 20, 32). It is thus essential to treat patients who suffer from documented hypothyroidism properly during pregnancy, with the administration of adequate doses of T₄ (levothyroxine) (12, 21).

Uterine rhythmic contractile activities are essential for normal labor and delivery. Inadequate contractions can lead to labor abnormalities and will often result in C-sections with associated surgical risks for both mothers and newborns (35). The existence of a large-conductance calcium-activated potassium channel (BKCa) is demonstrated in pregnant human myometrium and is even shown to be involved in uterine quiescence (2, 17, 22). A modification in BKCa conductance is related to a change in the functionality of uterine contractile response (16, 25).

Several investigative groups have analyzed the effect of thyroid dysfunction on pregnant uterine tissue and in particular have observed an effect of low levels of T₄ on uterine contractile activities in gravid rats (24, 30). Hypothyroidism has also been found to reduce calcium channel function in pregnant rat uterine tissue (30). Given that calcium influx is an essential component of excitation-contraction coupling in uterine smooth muscle, a downregulation of genes encoding for Ca²⁺ channel proteins may result in serious functional consequences during pregnancy and delivery. Indeed, Medeiros and Calixto (24) demonstrated that isolated myometrium ring responsiveness to various agonists, including acetylcholine, oxytocin, or divalent cation such as Ca²⁺, was increased significantly in the presence of hypothyroidism. However, no study has explored the effect of T₄ supplementation on uterine tissue responsiveness in pregnant women initially treated for documented hypothyroidism.

The present study aimed at analyzing the effect of T₄-Na treatment on human uterine smooth muscle properties. In a first series of experiments conducted in vitro, we compared specifically the spontaneous contractile activities in control and T₄-treated groups and observed major changes in the time course of contractile activity in human uterine strips recovered from pregnant women at term. Finally, we assessed the effect of acute addition of exogenous triiodothyronine (T₃) or T₄ on pregnant women myometrium in vitro.

MATERIALS AND METHODS

Study population. Patients admitted for an elective cesarean section were asked to participate in the study. The study was approved by the Institutional Ethics Committee for Research on Human Subjects at Université de Sherbrooke, Sherbrooke, QC, Canada (project no. 09-040, ClinicalTrials.gov identifier: NCT00939744), and all volunteers gave written informed consent. The inclusion criteria for the control group were 1) a gestational age between 37⁰/7 and 40⁰/7 wk, 2) a singleton gestation, 3) no labor and 4) signed, informed consent, and 5) for the T₄-treated group, a T₄ treatment during the entire pregnancy resulting from diagnosis before pregnancy. Exclusion criteria were

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Table 1. Demographic data of patients included in the in vitro study

<table>
<thead>
<tr>
<th></th>
<th>Control Group (n = 20)</th>
<th>T4-Treated Group (n = 8)</th>
<th>P Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, yr</td>
<td>Mean (range)</td>
<td>Mean (range)</td>
<td></td>
</tr>
<tr>
<td>30 (24–34)</td>
<td>29 (23–34)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td>38/2 (37–40)</td>
<td>38/2 (37–39)</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index</td>
<td>29 (18–47)</td>
<td>33 (24–48)</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>15</td>
<td>12.5</td>
<td>NS</td>
</tr>
<tr>
<td>Other medication</td>
<td>10</td>
<td>13</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means and range or percentage. T4, levothyroxine; NS, not significant. *Student’s t-test was performed, with P < 0.05 considered significant.

infections (chorioamnionitis, HIV, genital herpes, hepatitis B and C) and vaginal bleeding after the third trimester. The participants’ medical data were obtained from their medical file and a follow-up phone interview.

Sample collection. During the C-section, immediately after delivery but before maternal injection of oxytocin, biopsies of myometrium were excised from the upper lip of the lower uterine segment incision in the midline, as described previously (7). Once collected, tissue biopsies were placed in Krebs-Heinseleit physiological salt solution (Krebs) with the following composition (in mmol/l): 4.7 potassium chloride, 118 sodium chloride, 1.2 magnesium sulfate, 2.5 calcium chloride, 1.2 potassium phosphate, 25 sodium bicarbonate, and 11.1 glucose (Sigma-Aldrich, St. Louis, MO) and stored at 4°C and used within 8 h for contractility experiments or rapidly rinsed before snap-freezing in liquid N2 and stored at −80°C prior to biochemical analysis or preparation of subcellular fractions.

Western blot analysis. Subcellular fractions (cytosolic) were prepared from myometrium, fetal membranes, and placenta and were subsequently separated on 10% SDS-PAGE as described previously (8). For Western blot analysis, nitrocellulose membranes were blocked for 1 h with 5% nonfat dry milk in Tris-buffered saline with 0.1% Tween at room temperature. Blots were incubated overnight at 4°C with rabbit antiserum raised against DIO1 proteins (Abcam) and β-actin proteins. After washing, the membranes were incubated in a solution containing peroxidase-conjugated donkey anti-rabbit IgG antibody (Amersham). Protein labeling was detected using an enhanced chemiluminescence kit (Roche). Immunostainings were digitized and analyzed with Lab Image software version 2.7-2 (Kaplan).

Isolated organ bath experiments. Obtained biopsies were dissected in multiple longitudinal myometrial strips (measuring ~2 × 2 × 10 mm). Strips were cleansed of adherent tissue and mounted for isometric recordings under 2 g of resting tension in an organ bath system, as described previously (8, 9, 26). The organ baths contained 7 ml of Krebs solution maintained at 37°C, pH 7.4, and were gassed continuously with a mixture of 95% oxygen-5% carbon dioxide. Myometrial strips were allowed to equilibrate for 2 h after which a 30-min period was allowed to achieve spontaneous phasic contractions in the isolated organ bath. Passive and active tensions were assessed using transducer systems (Radnoti Glass Technology, Monrovia, CA) coupled to Polyview software (Grass-Astro-Med, West Warwick, RI) for facilitating data acquisition and analysis (7, 8). The effect of thyroid hormone was assessed using T3 and T4. T3 and T4 were dissolved in alkaline aqueous solution (pH = 8) and stored at −20°C for a maximal period of 1 mo. T3 and T4 were added separately in the organ bath in a cumulative manner at concentrations of 1 μM at 30-min intervals. Iberiotoxin (IbTx) and barium chloride (BaCl2) (Sigma-Aldrich, St. Louis, MO) were dissolved in water and stored at −20°C, whereas lemakalim (SmithKline Beecham, Herts, UK) stock solution (10 mM) was prepared in ethanol and stored at 4°C. Two sets of control experiments were performed as follows: control 1, strips exposed to Krebs’ solution only for ≤4 h, control 2, strips exposed to vehicle (alkaline aqueous solution or ethanol).

Data analysis and statistics. The amplitude, time to peak, duration of phasic contractions at 90% of relaxation (time required for the strip to relax to 90% of the maximal contraction), frequency, and area under the curves (AUC) for isometric tension studies were calculated on raw recordings from independent myometrial strips. The effect of T3 and T4 was compared with controls by calculating the contraction parameters at each 30-min interval and by normalizing the results as a percentage of the contraction parameters obtained in the 30-min basal activity period. Consecutive activities were quantified for each experimental condition using Sigma Plot 11.0 (SPSS, Chicago, IL). Data were distributed normally, and therefore, they were analyzed with parametric tests. Statistical analysis included paired t-test and Student’s t-test. Differences were considered significant when P < 0.05.

RESULTS

Population profile. The study group was composed of 28 healthy Caucasian pregnant women who underwent cesarean delivery between 37/2 and 40/2 wk of gestation (38/2 mean wk of gestation for the control group and 38/2 mean wk of gestation for the T4-treated group). Women in the control group had a mean age of 30 yr and a mean BMI of 29, whereas in the T4-treated group mean age was 29 yr with a mean BMI of 33 (Table 1). Indicators for cesarean section included previous cesarean sections (n = 12, n = 6), placenta previa (n = 3, n = 1), and breech position (n = 5, n = 1) for both control and T4-treated groups, respectively. Dosage of T3, T4, and TSH were not available for all patients in the medical database. Nevertheless, data concerning the T4-treated patients are presented in Table 2. Note that no dose adjustment was performed by the patient’s physician during the pregnancy, even if a TSH dosage was made (Table 2). Given that this study at the outset consisted of a fortuitous observation on already recruited patient, the present protocol approved by the Ethics Committee did not allow us to add blood assays prior to the cesarean sections.

Differences in contractile activities between T4-treated and control groups. Figure 1 displays typical recordings from myometrial strips obtained from either T4-treated or control pregnant women (Fig. 1, A and B). In vitro contractile activities from the T4 group (Fig. 1A) consistently displayed phasic contractions of larger amplitude and longer duration compared with contractile activities in the control group (Fig. 1B). Thus, these data have unmasked a modification of the pattern of uterine contractions in T4-treated group. Figure 1C illustrates a typical recording of a

Table 2. Thyroid status of patients under T4-treated group recruited in the in vitro study

<table>
<thead>
<tr>
<th>Patients</th>
<th>Doses of T4-Na, mg per OS</th>
<th>TSH Values, mIU/l (Normal Range: 0.35–3.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st Trimester</td>
</tr>
<tr>
<td>1</td>
<td>0.237</td>
<td>2.09</td>
</tr>
<tr>
<td>2</td>
<td>0.050</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>0.250</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>0.075</td>
<td>0.74</td>
</tr>
<tr>
<td>5</td>
<td>0.125</td>
<td>0.51</td>
</tr>
<tr>
<td>6</td>
<td>0.075</td>
<td>1.96</td>
</tr>
<tr>
<td>7</td>
<td>0.150</td>
<td>2.74</td>
</tr>
<tr>
<td>8</td>
<td>0.300</td>
<td>ND</td>
</tr>
<tr>
<td>Average</td>
<td>0.158 ± 0.033</td>
<td>1.61 ± 0.42</td>
</tr>
</tbody>
</table>

Average values are means ± SE. ND, not determined. *Lower than normal values, which represent hyperthyroidism.
Uterine strip obtained from a woman in the control group, which was stimulated by addition of 1 μM T₃ in the organ bath. The observed contractile pattern was relatively similar to that observed in strips from T₄-treated women, namely phasic contractions of longer duration and of slightly lower frequency.

Quantification of mean contractile parameters. In uterine strips from T₄-treated women, phasic uterine contractions of larger amplitude (+77%) were observed (Fig. 2A), along with similar time to peak (Fig. 2B), prolonged duration of contractions (+138%; Fig. 2C), and reduced frequency (−55%; Fig. 2D), compared with contractile values recorded in the control group (Fig. 2, A–D).

Addition of acute T₃ and T₄ on myometrium strips. No significant difference in relaxing effect was observed between time and vehicle controls (P = 0.310, P = 1.00). Figure 3 reports the quantification analysis following acute addition of 1 μM T₃ on contraction parameters. There was no acute effect of 1 μM T₃ on either amplitude (Fig. 3A) or time to peak (Fig. 3B) compared with the basal activity in uterine strips from the control group. However, there was a significant increase in the duration at 90% relaxation (Fig. 3C) with T₃ along with a net decrease in the frequency (Fig. 3D) of the contractile events recorded in vitro. The chosen concentration of T₃ used was determined according to the previously established concentration-response curve (0.1–1 μM) (data not shown). On the other hand, when concentrations of ≤1 μM of T₄ were added to the isolated organ bath, no effect was observed on contractile parameters, except for the frequency of contractions, where a significant decrease was noted (Fig. 4). Figures 3 and 4 show overlaps on contraction duration and frequency that can be explained by the intervariability of patients.

Western blot analysis of uterine tissues. Using a primary antibody raised against deiodinase type 1 (DIO1), an immunoreactive band was detected consistently at 78 kDa (Fig. 5A). This band was detected mainly in the cytosolic fractions from myometrium, fetal membranes, and placenta from control women but was barely detected in the myometrial cytosolic fraction from T₄-treated women (Fig. 5A, top). Quantitative analysis revealed that the DIO1-immunoreactive signal was significantly lower in placenta fractions from the T₄-treated group compared with the control group (P = 0.009). Although a similar tendency can be quantified in the myometrium and the fetal membranes, P values do not achieve significance (P = 0.057 and P = 0.240, respectively; Fig. 5B). Together, these data suggest that DIO1 was expressed in the control tissues but was consistently downregulated in the tissues recovered from the T₄-treated patients.

Modification of contractile responses using K⁺ channel blockers. To modify the amplitude and time course of phasic contractions, specific (IbTx) and nonspecific K⁺ channel blockers (BaCl₂) have been used. The idea was to trigger contractile activities similar to those observed upon thyroid hormone treatments (as reported in Fig. 1, A and C). Following a 30-min recording of basal activity (Fig. 6A), cumulative addition of 50–100 nM IbTx a induced positive effect on the amplitude of the phasic contractions from control uterine tissue. Moreover, concomitant addition of IbTx and 2 mM BaCl₂ induced long-lasting contractions with the appearance of a high-amplitude event followed by a sustained plateau. Data analysis was performed on typical recordings from this set of experiments. Upon additions of IbTx, there was a significant increase in the AUC (+58%; Fig. 6B). Hence, BaCl₂ further increased the AUC (+556%). Consequently, K⁺ channel blockers reduced K⁺ conductance of the myometrial cell membrane and indirectly reproduced the T₄-treated group myometrial contractile activity (Fig. 1A). In another set of experiments, 2 mM BaCl₂ alone had a significant effect on the AUC, whereas this activity was abolished upon extracellular calcium removal (Fig. 6B, right bars of the histogram).

Figure 7A illustrates a typical recording following the 30-min basal activity period and after addition of 1 μM lemakalim, a KATP potassium channel opener. Lemakalim, which is known to activate KATP potassium channels, hyperpolarized the myometrial membrane potential and abolished the contractile activity (25). This effect was reversed upon washout of the pharmacological compound. Hence, addition of 2 mM BaCl₂ alone had a significant effect on the amplitude and duration of the repetitive con-
tactions. Alternatively, concomitant addition of BaCl₂ and 1 μM lemakalim induced a high-frequency rhythmic contractile activity. Quantitative analysis displayed in Fig. 7 demonstrates a significant decrease in the mean AUC (−91%) upon addition of 1 μM lemakalim (P = 0.04), whereas 2 mM Ba²⁺ had a major positive effect on the AUC, which is likely related to a decrease in K⁺ conductance of the myometrial cell plasma membrane (Fig. 7B). Altogether, these results suggest that modification of the K⁺

Fig. 2. Modifications in uterine contractile parameters in uterine tissues of control and levothyroxine-treated pregnant women (control and T₄-treated groups). Uterine strips (n = 32) from both groups were mounted into isolated organ bath and spontaneous contractile activities recorded. Depicted are mean changes in amplitude (A), time to peak (B), duration to 90% relaxation (C), and contraction frequency (D). Data are means. *P < 0.05 using Student’s t-test. ●, Control group; ■, T₄-treated group.

Fig. 3. Acute effect of T₃ on uterine contraction parameters in tissues of control pregnant women (control group). Mean effects of 1 μM T₃ on amplitude (A), time to peak (B), duration to 90% relaxation (C), and contraction frequency (D) (n = 24). Strips were exposed to T₃ 10 min before the analyzing period. The enhanced duration of contractile events and lower frequency rate likely resulted from a nongenomic effect. Values are means. *P < 0.05 using a paired t-test. ●, Basal activity (for each parameter); ■, 1 μM T₃, as clearly defined.
conductances largely modified the time course of the myometrial contractile activity.

**DISCUSSION**

This study demonstrates clearly that myometrial contractile activities in vitro are modified significantly in the T4-treated group of pregnant women during their pregnancy compared with the standard contractile activities recorded from the control group. Indeed, this is an original observation-driven project in which significant modifications of contractile properties were delineated in myometrial strips from pregnant women treated by T4. Results also demonstrate that upon acute addition of T3 in vitro, but not with addition of T4, the duration of contractile events was increased. Thus, T3 addition was able to partially mimic the effects of the chronic treatment.

**Physiological impact of T4 supplementation on uterine mechanical properties.** In the 1980s, T3 nuclear receptors were identified in uterine nuclear fractions in both rats and humans (11, 18, 27). Ever since this discovery, the mode of action of T4 and T3 has been delineated in a number of organs. It has been proposed that T4 and T3 may control ionic conductances by genomic and nongenomic effects (3). For instance, it has been reported that T3 increases Na-K-ATPase activity, Na+/H+ exchanger, and membrane channel insertions (6). Moreover, T3 and T4 display structural similarities with amiodarone, a potent class III anti-arrhythmic agent known for its pharmacological properties on K+ channels that, among other things, controls the time course of mammalian cardiac action potential (19, 29, 31). The current results obtained in vitro in the presence of T3 (Fig. 3) suggest a putative effect of T3 on K+ conductance in uterine muscle cells, whereby changes in macroscopic ionic conductance could control the membrane potential and even amplitude and kinetics of contractile activities in myometrial strips (36).

**Chronic T4 treatment could be related to a reduction in K+ conductance.** We have shown that addition of IbTlx, which specifically blocks BKCa channels, significantly modified the amplitude and AUC of the basal activity of control strips. Moreover, BaCl2, which permeates through Ca2+ channels and blocks K+ channels, partially mimics the typical patterns consistently obtained with uterine biopsies from T4-treated patients. Despite the fact that we have not ruled out other putative intracellular modifications, such as changes in regulatory contractile proteins, uterine biopsies from T4-treated women display a decrease in their surface membrane K+ conductance. In contrast, lemakalim, a KATP channel activator that likely hyperpolarizes the human uterine smooth muscle cells and abolishes the phasic contractions,
represents a strong tocolytic, whereas barium enhances the amplitude and prolongs duration of contraction. Hence, lemakalim partially counterbalances the potent inotropic effects induced by \( \text{BaCl}_2 \). The tocolytic effect of lemakalim is concordant with previous data from the literature (15). Furthermore, barium significantly enhances the amplitude and increases duration of phasic contractions. Altogether, our data suggest that modifications of \( K^{\text{Ch}} \) conductance alter the contractile pattern of human uterine strips, which would explain indirectly the abnormal time course of uterine-repetitive contractions in uterine biopsies from \( \text{T}_4 \)-treated women. Bearing in mind that BKCa and KATP potassium ion channels are involved in the control of membrane voltage, action potential duration, and time course of contractile events of human myometrial strips at term (1, 34), a reduced \( K^{\text{Ch}} \) conductance would result in action potentials of longer duration, which would induce a delayed relaxation and enhanced duration of the overall contractile events.

Our current result would encourage us to assess the putative effects of \( \text{T}_4 \) and \( \text{T}_3 \) on the time course of action potentials recorded from myometrial strips using the classical microelectrode technique (10, 28) to confirm whether or not such changes in \( K^{\text{Ch}} \) conductance could be induced by \( \text{T}_3 \) and \( \text{T}_4 \) and are indeed responsible for the observed prolonged contractile activities or alterations thereof. An alternative strategy would be to quantify the expression of various \( K^{\text{Ch}} \) channels (\( \alpha \)- and \( \beta \)-subunits) or regulatory proteins of the contractile machinery using quantitative PCR under various experimental conditions and physiological states (13).

The original observation made on uterine strips from chronically \( \text{T}_4 \)-treated pregnant women at term is rather challenging. According to Parija et al. (30), a decrease in amplitude and an increase in frequency of uterine contractions was demonstrated (in vitro) in a model of hypothyroid pregnant rates. In our study, we demonstrate and report the opposite effect since the contractile activity patterns from the \( \text{T}_4 \)-treated group were characterized by larger amplitude, increased duration, and lower frequency. These observations support our hypothesis that the contractile activity patterns delineated in the \( \text{T}_4 \)-treated group were likely due to the therapy and not to an underlying hypothyroidism.

In vitro \( \text{T}_4 \) treatment affects only frequency of uterine contractions. Our data show that acute \( \text{T}_4 \) treatment reduced frequency of phasic contractions. In contrast, we found no effect of acute \( \text{T}_4 \) treatment on either the amplitude or duration of contractions. This unexpected result may be explained by the short period of time allowed for the transformation of \( \text{T}_4 \) in \( \text{T}_3 \). Hence, it would take time for \( \text{T}_3 \) to activate the genomic, transcriptomic, and proteomic machineries to produce specific membrane or contractile regulatory proteins. Thus, \( \text{T}_3 \) could be responsible for the present effect. Indeed, the free \( \text{T}_3 \), free \( \text{T}_4 \), and TSH plasma concentration ranges in the first, second, and third trimesters were, respectively, 1.92–5.86, 3.2–5.73, and 3.3–5.18 pM/l for \( \text{T}_3 \), 12–19.45, 9.48–19.58, and 11.32–17.7 pM/l for \( \text{T}_4 \), and 0.6–5.0, 0.44–5.78, and 0.74–5.7 iU/ml for TSH (23).

Underlying enzymology. Western blot analyses were thus performed to assess the presence of DIO1, a selenoenzyme that...
converts T₄ to T₃. The presence of DIO1 was detected in myometrium, fetal membranes, and placenta in both control and T₄-treated groups. The immunoblot analysis demonstrated that DIO1 was decreased in the myometrium from the T₄-treated group, thus suggesting that T₄ was poorly transformed in T₃ in uterine tissues from the T₄-treated groups. Despite the presence of uterine DIO1 in both groups, we cannot rule out the presence of type 3 deiodinase, known to be present in myometrium and endometrium, which could transform T₄ in reverse rT₃ in women, as reported previously (14). The prevailing presence of reverse T₃ would also explain the fact that acute addition of T₄ had no effect on contraction parameters, except on frequency, as demonstrated in our in vitro analysis. Considering that the TSH values were normal and that DIO1 expression was reduced in the pregnant myometrium from the T₄-treated group (Table 2 and Fig. 5), it would be of prime interest to further assess the complete thyroidian status during pregnancy to ensure a tighter followup of patients prior delivery.

Clinical considerations. The T₄-treated population had a BMI of 33, representing a variation compared with the control group (BMI = 29). A previous study demonstrated clearly that the basal uterine contractility of obese women is decreased, with their myometrium (BMI ≥ 30) contracted with less force (lower amplitude), and the Ca²⁺ influx of these women is decreased (37). Therefore, we could consider that the high mean BMI in our T₄-treated patients did not influence the results because of the observed higher amplitude and duration in this group, which does not correlate with reported effects on myometrial contractions of obese women. In view of the challenging observations made, it would thus be of interest to set a clinical study to further extend the present data and to confirm these challenging results.

In conclusion, the present study clearly demonstrates modifications in the contractile pattern of myometrial strips at the end of pregnancy retrieved from chronically T₄-treated pregnant women. The present data clearly underscore the potential relevance of studying the clinical impact of T₄ administration during each pregnancy trimester as well as on labor outcomes in light of the fact that hypothyroidism increases the risks for the fetus during pregnancy. Bearing in mind that T₄ treatment is acknowledged to be essential for fetal growth and development, a better management of such treatment at the end of pregnancy may be helpful for the clinical management of labor in this particular group of women.

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DISCLOSURES
The authors declare no conflicts of interest, financial or otherwise.

AUTHOR CONTRIBUTIONS
S.C., J.-C.P., and E.R. contributed to the conception and design of the research; S.C. performed the experiments; S.C. analyzed the data; S.C., J.-C.P., S.B., D.B., and E.R. interpreted the results of the experiments; S.C. prepared the figures; S.C. drafted the manuscript; S.C., S.B., D.B., and E.R. edited and revised the manuscript; S.C., J.-C.P., D.B., and E.R. approved the final version of the manuscript.

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