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 effects 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 T₃-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 T₄. 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.

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 37⁰⁰⁰ and 40⁰⁰⁰ 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...
Table 1. Demographic data of patients included in the in vitro study

|                          | Control Group (n = 20) | T4-Treated Group (n = 8) | P Value
|--------------------------|------------------------|--------------------------|---------
| Maternal age, yr         | 30 (24–34)             | 29 (23–34)               | NS      
| Gestational age, wk      | 38(7/5 (37–40)        | 38(7/5 (37–39)          | NS      
| Body mass index          | 29 (18–47)             | 33 (24–48)               | NS      
| Smoking, %               | 15                     | 12.5                     | NS      
| Other medication         | 10                     | 13                       | NS      

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

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, mU/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>
</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 T3 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-
tractions. Alternatively, concomitant addition of BaCl$_2$ and 1 μM lemakalim induced a high-frequency rhythmic contractile activity. Quantitative analysis displayed in Fig. 7B demonstrated a significant decrease in the mean AUC (−91%) upon addition of 1 μM lemakalim ($P = 0.04$), whereas 2 mM Ba$^{2+}$ 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$^+$

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**Fig. 2.** Modifications in uterine contractile parameters in uterine tissues of control and levothyroxine-treated pregnant women (control and T$_4$-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; ■ T$_4$-treated group.

**Fig. 3.** Acute effect of T$_3$ on uterine contraction parameters in tissues of control pregnant women (control group). Mean effects of 1 μM T$_3$ on amplitude (A), time to peak (B), duration to 90% relaxation (C), and contraction frequency (D) ($n = 24$). Strips were exposed to T$_3$ 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 T$_3$, 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 mimick 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 lbTx, which specifically blocks BK\(_{Ca}\) channels, significantly modified the amplitude and AUC of the basal activity of control strips. Moreover, BaCl\(_2\), which permeates through Ca\(^{2+}\) 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 K\(_{ATP}\) 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 BaCl₂. 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\textsubscript{Ca} 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 T4-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\textsubscript{Ca} 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 T\textsubscript{4} and T\textsubscript{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\textsuperscript{+} conductance could be induced by T\textsubscript{3} and T\textsubscript{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\textsuperscript{+} channels (α- and β-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 T\textsubscript{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 T4-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 T4-treated group were likely due to the therapy and not to an underlying hypothyroidism.

In vitro T\textsubscript{4} treatment affects only frequency of uterine contractions. Our data show that acute T\textsubscript{4} treatment reduced frequency of phasic contractions. In contrast, we found no effect of acute T\textsubscript{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 T\textsubscript{4} in T\textsubscript{3}. Hence, it would take time for T\textsubscript{3} to activate the genomic, transcriptomic, and proteomic machineries to produce specific membrane or contractile regulatory proteins. Thus, T\textsubscript{3} could be responsible for the present effect. Indeed, the free T\textsubscript{3}, free T\textsubscript{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 T\textsubscript{3}, 12–19.45, 9.48–19.58, and 11.32–17.7 pM/l for T\textsubscript{4}, and 0.44–5.0, 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

![Image](http://ajpendo.physiology.org/doi/10.1152/ajpendo.00346.2012/fig6/6.png)

Fig. 6. Effect of iberiotoxin (IbTx) and barium chloride (BaCl₂) on the repetitive contractile activity of human uterine strip. A: typical activity of a human myometrial strip recovered from a pregnant woman (control group). Following a 2-h equilibration period, the basal activity was recorded prior to sequential additions of 50 and 100 nM IbTx and 2 mM BaCl₂. Note the changes in amplitude and duration of the phasic contractions. B: quantitative analysis of normalized area under the curve (AUC) upon addition of K\textsuperscript{+} channel blockers. Note that the right bar histograms display the results from a distinct set of experiments in which 2 mM BaCl₂ was used in the presence of 2.2 mM or in the absence of CaCl₂. Data represent means ± SE (n = 12; *P < 0.05).
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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Fig. 7. Effect of lemakalim (Lem) and BaCl₂ (Ba²⁺) on the repetitive contractile activity from human uterine strip. A: typical recording of a human myometrial strip recovered from a pregnant woman (control group). 1 μM Lem, a K⁺ₐ₆₅₉₃ channel opener, abolished the contractile activity. Following a 45-min washout period (W), 2 mM BaCl₂ triggered long-lasting phasic contractions, whereas concomitant addition of 2 mM Ba²⁺ + 1 μM Lem induced a repetitive activity of high frequency compared with the basal activity. B: quantitative analysis of the K⁺ channel opener and blocker on normalized AUC attests that changes in K⁺ conductance indirectly modified the human myometrial activity. Data represent means ± SE (n = 12; *P < 0.05).
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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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