Inhibitory effect of leptin on human uterine contractility in vitro

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KEY WORDS
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Objective: The purpose of this study was to investigate the effects of leptin on human uterine contractility in vitro.

Study design: Biopsies of human myometrium were obtained at elective cesarean section (n = 18). Dissected myometrial strips suspended under isometric conditions, undergoing spontaneous and oxytocin-induced contractions, were exposed to cumulative additions of leptin in the concentration range of 1 nmol/L to 1 μmol/L. Control strips were run simultaneously. Integrals of contractile activity were measured using the PowerLab hardware unit and Chart v3.6 software.

Results: Leptin exerted a potent and cumulative inhibitory effect on spontaneous and oxytocin-induced contractions compared to control strips. The mean maximal inhibition values were as follows: 46.79 ± 5.13% (n = 6; P < .001) for spontaneous contractions and 42.32 ± 3.69% (n = 6; P < .001) for oxytocin-induced contractions. There was an apparent reduction in both frequency and amplitude of contractions.

Conclusion: This physiologic inhibitory effect of leptin on uterine contractility may play a role in the dysfunctional labor process associated with maternal obesity, and the resultant high cesarean section rates.

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Obesity is rapidly becoming a major health problem in the developed world. Apart from the general adverse health implications of obesity, it is now apparent that obesity in pregnancy is linked to a significant increase in complications such as failure of the labor process, cesarean section, hypertensive disorders, gestational diabetes mellitus, and fetal macrosomia.1,2 There are numerous possible explanations for the association between obesity and failure of normal parturition, which include increased fat deposition in the maternal pelvis, fetal macrosomia, and an inadequate active second stage of labor. The possibility of altered metabolic modulation of myometrial smooth muscle in association with obesity has hitherto not been considered, despite the widely reported functional roles of secretory products of adipose tissue, and the emerging

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concept that these secretory products play an important role in the pathophysiology of obesity related complications.3

Leptin is a peptide with 167 amino acid residues that is secreted from adipose tissue under the control of the obesity gene,4 and is known to have regulatory effects on neuronal tissue, vascular smooth muscle, and nonvascular smooth muscle systems.5 Leptin concentrations rise significantly with increasing percentage body fat,6 and obese individuals have markedly increased leptin production.7 The role of leptin in human reproduction is not clear. While leptin is most likely involved in the regulation of ovarian function, oocyte maturation, embryo development and implantation,8 the role of leptin during pregnancy has not been established. The placenta is a major source of leptin production during pregnancy,9 and leptin may play a role in the pathophysiology of pre-eclampsia10 and hypertensive disorders of pregnancy.11 Leptin and leptin receptor genes are also expressed in human umbilical cord, fetal membranes, and uterine tissue.12

In the human13 as well as in rat14 and higher mammalian models,15 serum leptin levels are markedly elevated during pregnancy and demonstrate a significant decrease just before or at the time of parturition. Because of this, and because of the diverse metabolic functions associated with leptin, we hypothesized that leptin may modulate myometrial function and activity. The aim of this study was therefore to investigate the effects of leptin on human uterine contractility in vitro, on spontaneous contractions and those elicited by the agonist oxytocin.

**Material and methods**

**Tissue collection**

Biopsies of human myometrial tissue were obtained at elective cesarean section in the third trimester of pregnancy in the Department of Obstetrics and Gynecology, University College Hospital, Galway, Ireland. The biopsies were excised from the upper lip of the lower uterine segment incision in the midline, ie, upper portion of lower uterine segment. Ethical committee approval for tissue collection was obtained from the Research Ethics Committee at University College Hospital Galway and recruitment was by written informed consent. Once collected, all tissue biopsies were placed in Krebs-Henseleit physiologic salt solution (PSS) at pH 7.4 containing the following: 4.7 mmol/L potassium chloride, 118 mmol/L sodium chloride, 1.2 mmol/L magnesium sulphate, 1.2 mmol/L calcium chloride, 1.2 mmol/L potassium phosphate, 25 mmol/L sodium bicarbonate, and 11 mmol/L glucose (Sigma-Aldrich, Dublin, Ireland). Tissues were stored at 4°C and used within 12 hours of collection.

**Tissue bath experiments**

Longitudinal myometrial strips (measuring approximately 2 × 2 × 10 mm) were dissected free of uterine decidua and serosa and mounted for isometric recording under 2 g of tension in organ baths as previously described.16,17 The tissue baths contained 10 mL of Krebs-Henseleit PSS 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 at least 1 hour during which time the Krebs-Henseleit physiologic salt solution was changed every 20 minutes. After equilibration, a 30-minute period was allowed in order to achieve spontaneous phasic contractions, or, alternatively, contractions were stimulated by bath exposure of the strips to oxytocin (0.5 nmol/L). Leptin was then added to the tissue bath in a cumulative manner at bath concentrations of 1 nmol/L, 10 nmol/L, 100 nmol/L, and 1 µmol/L at 20-minute intervals. There were 2 sets of control experiments performed as follows: control 1. Strips exposed to either PSS only (for spontaneous contractions) or 0.5 nmol/L oxytocin; and control 2. Strips exposed to PSS and vehicle for leptin (spontaneous contractions) or 0.5 nmol/L oxytocin and vehicle for leptin. The effect of leptin and the respective controls were assessed by calculation of the integral from minimum of selected areas for each 20-minute intervals and expressed as a percentage of the integral obtained in the 20-minute period before any leptin addition using the PowerLab hardware unit and Chart v3.6 software (AD Instruments, Hastings, UK). The inhibitory effect of leptin was corrected for the reduction in the contractile activity observed in the vehicle control (control 2), and the effects of leptin were interpreted as the final additional relaxant effect. This value, when subtracted from 100% represents the mean maximum inhibition (MMI) of leptin on myometrial contractility. The MMI provided therefore represents the net final inhibition resulting from exposure to leptin, ie, after subtraction of any alteration observed in vehicle control (control 2) experiments.

**Drugs and solutions**

Leptin was purchased from Sigma-Aldrich. A stock solution (100 µmol/L) was made in 15 mmol/L hydrochloric acid and 7.5 mmol/L sodium hydroxide. Serial dilutions were made in deionized water on the day of experimentation. Fresh Krebs-Henseleit solution was made daily. A stock solution of oxytocin (1 mmol/L; Sigma-Aldrich) was prepared using deionized water. Serial dilutions were prepared in deionized water on the day of experimentation.

**Statistical analysis**

Comparisons of contractile effect, for each bath concentration of leptin, were performed using analysis of
variance (ANOVA) followed by Tukey HSD post hoc testing to determine significant differences among data groups. A $P$ value of $< .001$ was considered to be statistically significant. A paired sample $t$ test was used to compare spontaneous and oxytocin-induced vehicle controls to spontaneous and oxytocin-induced controls, respectively. The paired sample $t$ test showed that there was no significant difference between the spontaneous vehicle control and the spontaneous control ($P = .116$) and it showed that there was no significant difference between the oxytocin-induced vehicle control and the oxytocin induced control strips ($P = .164$). The statistical package SPSS for Windows version 11.0 (SPSS, Inc, Chicago, IL) was used for these statistical calculations.

Results

Myometrial biopsies were obtained from a total of 18 women who underwent cesarean delivery between 38 and 40 weeks of gestation (median gestation, 38 weeks). The reasons for cesarean section were previous cesarean section ($n = 15$), placenta praevia grade 4 ($n = 1$), breech presentation ($n = 1$), and previous pregnancy losses ($n = 1$). The mean maternal age at delivery was 32.5 years (range 22-41 years). The median parity value of the women at the time of delivery was 1 (range 0-3). All cesarean sections were carried out under regional anesthesia.

Leptin exerted an inhibitory effect on both spontaneous and oxytocin-induced contractions in human myometrium in vitro, in all strips, in comparison to control measurements. A representative recording of spontaneous myometrial contractions is shown in Figure 1A (control 1), and Figure 1B demonstrates a recording of spontaneous myometrial contractions treated with increasing concentrations of vehicle for leptin (1 nmol/L, 10 nmol/L, 100 nmol/L, and 1 μmol/L) (control 2). Spontaneous controls 1 and 2 were compared using a paired $t$ test and no significant differences were found between the 2 controls, $P = .116$. Figure 1C demonstrates the uterorelaxant effect of leptin on spontaneous contractions in myometrial tissue with an observed reduction in both amplitude and frequency. The results of the calculated integrals of contractile activity are provided in the Table. A mean maximal inhibition (MMI) of 46.794 ± 5.133% ($n = 6$) was recorded ($P < .001$). The reduction in contractility for spontaneous contractions was cumulative and attained statistical significance at a bath leptin concentration of 1 μmol/L, in comparison to control strips (with vehicle). This is demonstrated in Figure 2A, where a graphic representation of the effect of increasing concentrations of leptin on spontaneous myometrial contractions is shown.

The results obtained for oxytocin-induced myometrial contractility are similarly shown in Figure 3 and the
This is demonstrated in Figure 2B, where a graphic representation of the effect of increasing concentrations of leptin on oxytocin-induced myometrial contractions is shown.

The results from this study demonstrate that leptin acts as a potent inhibitor of myometrial contractility in vitro. This relaxant effect was observed at relatively low concentrations for in vitro experiments and was apparently cumulative in nature. Observing the raw data, it was evident that leptin reduced both amplitude and frequency, but for proper accuracy and analysis of
results, the integrals of contractile activity measured over a period of time were compared. The findings from these experiments are robust because of the fact that 2 separate series of control experiments were performed. The findings were similar for spontaneous and oxytocin-induced myometrial contractions. The conclusions are therefore reliable and reproducible, indicating a potent inhibitory effect of the peptide leptin. In addition, the leptin levels investigated in this study include the normal ranges of serum leptin measured in normal and obese women.\textsuperscript{19,20} Upon conversion from ng/mL to molarity, it is apparent that the normal levels of leptin in pregnancy are in the nanomolar range, increasing by a factor of more than 10 in obesity. This is fully covered by the range of doses used in these experiments.

It is well established that maternal obesity is a major mitigating factor against successful vaginal delivery, after allowing for all other variables. We, and other groups, have demonstrated that cesarean section rates for women with a body mass index (BMI) indicating obesity (ie > 30), or morbid obesity (>35), are of the order of 35% to 50%, which represents an odds ratio (OR) of 2 to 3 in comparison to women of normal BMI.\textsuperscript{1,2} Earlier reports have also outlined the significantly increased caesarean section rates associated with obesity.\textsuperscript{19,20} Possible reasons for this have included increased maternal deposition of adipose tissue resulting in physical obstruction to descent of the fetal presenting part, fetal macrosomia, and inadequate pushing in the second stage of labor. Our findings introduce the new dimension that obesity can result in altered metabolic modulation of human myometrial tissue, raising the possibility that leptin, which is increased significantly in association with obesity,\textsuperscript{6,21} contributes to inadequate contractility of the uterus during labor. These results provide a further explanation for the significant disparity that exists in successful vaginal delivery rates between women of normal BMI, and those who fall into the obese categories.

The physiologic importance of leptin in pregnant women is largely unknown. Leptin is increased in the maternal blood particularly in the second trimester, with a reduction in levels towards or at the time of parturition.\textsuperscript{13,22} Apart from its production in adipose tissue, leptin is also produced in the placenta.\textsuperscript{8,10} Leptin is known to have several mechanisms of functional activity linked to autonomic, cardiovascular, inflammatory, and endocrine pathways,\textsuperscript{4} aside from a possible direct effect via intracellular signalling mechanisms. It is therefore difficult to speculate in relation to mechanism of action of the uterorelaxant effect of leptin reported in this study.

Apart from future investigations relating to the mechanism of leptin-induced myometrial inhibition, there are other questions raised by this study. The experiments reported here were all performed in lower segment myometrium. While it is generally believed that the functional aspects of lower segment myometrium are similar to those of upper segment myometrium,\textsuperscript{23} this issue merits further investigation. The effects of leptin on nonpregnant myometrial contractility are also a topic for future study. These findings also raise the possibility that other peptides and bioactive agents released from the excessive adipose mass that occurs in obesity\textsuperscript{3} may modulate myometrial function.

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References


