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Altered Uterine Gene Expression in Lean and Obese Mice Following Maternal Oxytocin

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Abstract

Background: Obese women exhibit higher rates of failed labor inductions with oxytocin. To investigate the mechanisms underlying parturition dysfunction in obese populations, we examined the changes in uterine gene expression profiles in lean and obese mice at term, with and without maternal oxytocin administration.

Methods: Female C57BL/6 mice were fed either a high-fat or regular-lean diet for 6 weeks prior to conception and throughout pregnancy. At term, dams were given saline or oxytocin, with a second group of obese mice receiving high-dose oxytocin. Six hours later, uterine gene expression for 30 select transcripts associated with parturition (e.g. gap junctions, relaxation/contractility pathways, and oxytocin signaling) and obesity were analyzed by quantitative real time PCR.

Results: Lean and obese uteri, at baseline, showed differential gene expression patterns at term. Oxytocin significantly altered the expression of numerous myometrial transcripts associated with parturition (gap junctions, relaxation/contractility pathways, and oxytocin signaling). The expression of numerous oxytocin-responsive genes depended on the dams' body masses (lean vs. obese), with either blunted effects or no effects of oxytocin observed in obese mice vs. lean mice. Additionally, high-dose oxytocin did not consistently regulate parturition-related gene expression in obese uteri. In summary, gene expression patterns significantly differed in lean vs. obese uteri at term in the presence and absence of maternal oxytocin. Lean uteri were more responsive to oxytocin than obese uteri, even at higher doses of oxytocin.

Conclusions: These findings support that blunted oxytocin responsiveness in obese uteri may contribute to obesity-related labor dysfunction.

Keywords: Labor Induction; Obesity; Oxytocin; Parturition; Uterine Contraction

Introduction

Labor is a complex physiological process requiring changes in the structure and function of the uterus and cervix. The transition from uterine quiescence to the onset of contractions results from increased levels of contractile stimulators and loss of uterine relaxation [1]. Phasic and coordinated contractions are pertinent for successful labor outcomes. Despite multiple studies attempting to understand this process, the precise mechanisms of labor onset are poorly understood. In the US, approximately 30% of women undergo labor induction [2]. In clinical practice, oxytocin (OXT), a neuropeptide, is

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exogenously administered to induce and augment labor [3, 4]. OXT binds to OXT receptors (OXTRs) to initiate a cascade of events resulting in gene and protein expression changes, as well as calcium release leading to cytoskeletal changes that culminate in myocyte contractions [5].

Obese patients experience delayed labor initiation, protracted courses of labor and higher rates of failed inductions [6-10]. In addition, obese women may require higher doses of OXT and longer durations of OXT for labor augmentation [4, 11-13]. These complexities may lead to more cesarean deliveries and increased maternal and neonatal morbidities and mortality among obese women [7, 10, 14-16]. The biologic mechanisms responsible for parturition dysfunction and reduced OXT responsiveness among obese women are not completely understood, creating an unmet need for improving the labor process for this 'at-risk' group. To our knowledge and based on a search of the literature, no prior study examined a panel of genes associated with parturition (including gap junctions and uterine relaxation and contraction pathways) in lean and obese uteri at term, with and without maternal OXT administration.

Based on the influence of obesity on parturition and OXT responsiveness, we hypothesized that maternal obesity (vs. normal weight) would alter gene expression patterns in the myometrium at baseline and following maternal OXT treatment. To test this hypothesis, we examined the effect of maternal obesity on myometrial gene expression and responsiveness to oxytocin (vs. saline) using targeted quantitative real time PCR (qPCR), focusing on gene expression associated with parturition (gap junctions, uterine contractility, and relaxation, and OXT signaling) and obesity. In addition, to mimic higher doses of OXT administered to obese women, we also examined the effect of high dose vs. regular dose OXT on uterine gene expression in obese mice.

Methods

Ethics Approval

The Institutional Animal Care and Use Committee (IACUC) of the Feinstein Institutes for Medical Research reviewed and approved the animal studies (IACUC #2015-053) prior to animal experimentation. All animal experimentation was in accordance with the guidelines for animal care provided by the National Institutes of Health. No human experimentation was performed.

Mouse Model of Pregnancy

After acclimation to normal environmental conditions with 12hrs light and dark cycles, female Mus musculus (mice, C57BL/6, 4-5 weeks old, from Charles River) were fed either high-fat diet (HFD, D-12492, Research Diets Inc) or standard regular chow diet (D12450B, Research Diets Inc). The HFD consisted of 60% of Kcal as fat and 20% Kcal as protein; the regular diet consisted of 10% of Kcal as fat and 20% of Kcal as protein. After 6 weeks on their respective diets, females (10-11 weeks old) were mated with regular diet-fed C57BL/6 males (10-15 weeks old, Charles River). Timed matings were set up in the late afternoons approximately twice weekly during proestrus (based on the assessment of external female genitalia and microscopic evaluation of vaginal cell morphology following lavage [17], using male-urine synchronized cycles). The following morning (before 7am) males were removed and females were checked for sperm plugs or the presence of sperm in vaginal lavage and for vaginal cell morphology (determined as the first day of gestation; gestational day (GD) 0.5). Females were weighed twice weekly to confirm timed pregnancies. Pregnant mice were maintained on their respective diets throughout the gestation. This model of gestational obesity using pre- and post-conception administration of the HFD is similar that previously described by Chang et al using C57BL/6 mice [18]; this HFD model in female C57BL/6 mice was chosen because it induces obesity, lipid derangements and hypercholesterolemia [19], mimicking obesity with lipid dysfunction.

On GD18.5 (at term, the mean gestation length for C57BL/6 mice is 19 days), mice (n=6-7 per group) were administered either saline (100µl) or 1U oxytocin (OXT, JHP Pharmaceuticals, Inc) administered in 100µl subcutaneously (s.c.) every 30 min for 2 hours (cumulative dose of 5U/mouse (equivalent to 0.334mg/kg), regular [reg]-dose). This dosing method was chosen based on veterinary OXT use in mice [20] and to avoid invasive pump implantation in term-pregnant mice. This dose was based on [21] and our prior studies showing that 5U OXT per day (n=7 dams/group) did not induce labor when compared to saline (all mice delivered within 15-20 hours of initial treatment [i.e. the expected time-frame]) and to avoid the delay in delivery observed with low dose maternal OXT [21]. On GD18.5 (at term), another set of obese dams (n=6) were given double the dose of OXT (2U/100µl s.c. every 30 min for 2 hours (cumulative dose of 10U/mouse, high [hi]-dose OXT)) to mimic a higher dose given to obese women. Six hours after the first OXT dose, dams were euthanized by CO2 asphyxiation and exsanguination; pups were delivered by C-section and uteri were collected. Note: none of the dams delivered within 6 hours of saline or OXT administration. Myometrial tissue was prepared as previously described [22]. Briefly, both uterine horns were dissected, the uterine tissue closest to the cervix was removed, opened and rinsed with PBS. Placental tissue was removed from the uterine tissue and care was taken to avoid the inclusion of decidua basalis. Myometrial tissue was immediately frozen in liquid nitrogen and then stored at -80◦C for RNA isolation.

RNA Isolation and Gene Expression Studies

RNA was isolated using the RNeasy Universal Plus Mini

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Kit (Qiagen), which included a genomic DNA elimination step. RNA quality was analyzed using the Nanodrop spectrophotometer and the Bioanalyzer (Agilent Technologies Genomics). Total RNA samples with OD260:280 and OD260:230 ratios >1.9 and RIN values > 9.3 were used for analyses.

Experiment 1. Assessment of Uterine Contractility/ Relaxation Pathways in Lean vs. Obese Dams ± OXT

A panel of 30 mRNA transcripts (Supplementary Table 1, see section of supplementary data given at the end of this article) was selected to assess obesity, gap junction-related pathways, and parturition-related pathways (contractility and relaxation, OXT signaling), as previously described in the literature. Targeted qPCR was performed using 6-7 uteri/group (lean±vehicle or OXT and obese±vehicle or OXT). Briefly, genomic DNA-free RNA (1µg per uterus) was reverse transcribed to double stranded cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) with the following temperature cycles: 25°C for 10 min, 37°C for 120 min and heat inactivation at 85°C for 5 min. Real time PCR/qPCR using synthesized cDNA, specific primers and the Roche Universal Probe Library was performed using the Eurogentec qPCR MasterMix Plus (AnaSpec, Inc) (see Supplementary Table 1) and the Roche LightCycler 480 (each transcript was run in triplicate) using the following cycling programs: 50°C for 2 min and 95°C for 10 min for 1 cycle, followed by 45 cycles of 95°C for 15 sec and 60°C for 75 sec. Data were analyzed for relative changes in gene expression (using Roche LightCycler software and Microsoft Excel) and reported as fold-changes by the $2^{-\Delta\Delta Ct}$ method using Gapdh for normalizing transcript levels. Note: *Gapdh* transcripts were shown to be stable across mice, conditions, and experimental days.

Experiment 2. Assessment of OXT Dosing in Obese Dams

The effect of high (hi)-dose oxytocin administration to obese dams (n=6-7 per group; obese+vehicle, obese+reg-dose OXT, and obese hi-dose OXT) on the expression of 30 specific genes implicated in parturition-related pathways (contractility and relaxation, OXT signaling) and obesity was analyzed by qPCR, as described above.

Statistical Analysis

Sample sizes: Sample sizes for the 2x2 factorial design (experiment 1) and the dose escalation study (experiment 2) were based on published studies using OXT for studying labor induction and uterine contractility in mice [21, 23].

Experiment 1. Assessment of Uterine Contractility/ Relaxation Pathways in Lean vs. Obese Dams ± OXT: Based on the study design two-way analysis of variance (ANOVA) was used to examine the association between expression levels and group (lean, obese) and OXT (no, yes) (2x2 factorial design). The interaction between group (lean vs. obese) and treatment (no OXT, OXT) was included in the model. If the interaction was significant, then the following pre-specified comparisons were examined within the ANOVA model: lean mice ± OXT; obese mice ± OXT; and lean vs. obese mice (–OXT). For these comparisons, a Bonferroni adjustment was used, such that $p<0.0167$ was considered significant. If the interaction between group and OXT was not significant, it was removed and the main effects of group (lean, obese) and treatment (no OXT, OXT) were examined; $p<0.05$ was considered significant. It should be noted that the purpose of the interaction term is to examine whether the change in expression levels with treatment (no OXT, OXT) differed depending on group (lean/ control, obese). In those models where the interaction was not significant, the interaction term was removed and only the effects of treatment and group on expression levels were examined.

Experiment 2. Assessment of OXT Dosing in Obese Dams: Based on the study design analysis of variance, (ANOVA) was used to examine the association between expression levels and OXT (no, OXT, regular dose OXT and high dose OXT; n=6/group). If there was a significant effect of OXT, pairwise comparisons were carried out within the ANOVA model. A Bonferroni adjustment was used, such that $p<0.0167$ was considered significant for these comparisons.

For experiments 1 and 2, the $\log_2$ transformation was used to better meet the assumptions of the ANOVA model. Log_2 transformed mRNA expression data more closely follows a normal distribution than untransformed expression data. For all genes in experiments 1 and 2 vehicle-treated lean mice (who did not receive OXT) were the reference group (i.e. all levels were divided by the mean expression level in this group). Summary statistics are given as least squares means and their associated 95% confidence intervals determined from the ANOVA model and then transformed back to non-log scale expression levels (fold-change in expression).

Results

Maternal weight gain and litter assessments

The average weights of lean and obese dams at term were statistically different, with obese mice weighing approximately 33% more (44.1±5.7g [obese] vs. 33±3.6g [lean], $p<0.0001$). Also, pup weights differed, with heavier pups in the obese group when compared to the lean group (0.98±0.2g [obese] vs. 0.87±0.1g [lean], $p=0.0005$). Litter sizes were comparable in lean and obese groups (7.5±1.7 vs. 8.4±2.5, $p=0.3$).

Differential Expression of Uterine Contractility/ Relaxation Pathways in Lean vs. Obese Dams following Maternal OXT Administration

Using a 2x2 study design in lean and obese mice, we

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examined the effects of maternal OXT administration (or vehicle) on a panel of genes associated with OXT signaling, parturition pathways (e.g. uterine contractility and relaxation pathways), and obesity (See Supplementary Table 1 for the full list of genes). Among the 30 genes analyzed, 11 genes showed significant interactions – i.e. the effect of OXT on gene expression depended on whether the animals were lean or obese (Table 1 and Supplementary Table 2 for full data set). These genes included *Edn1, Gucy1a3, Gucy1b3, Kcnq1, Mapk1, Mylk, Mypt3, Npr1, Pgrmc1, Pgrmc2*, and *Prkca* (Table 1). In lean mice, the uterine expression of all 11 genes was significantly reduced by maternal OXT administration, whereas only *Mypt3* and *Npr1* expression was significantly reduced in obese uteri. However, greater effects were observed in lean mice (Table 1). Finally, the expression of *Edn1, Kcnq1, Npr1*, and *Prkca* was significantly higher in lean vs. obese uteri, irrespective of OXT treatment (Table 1).

For the remaining genes, where no significant interactions were observed, the main effects of group (lean vs. obese) and treatment (saline vs. OXT) were analyzed. Among these 19 genes, 15 showed significant differences ($p<0.05$) (Table 2). Almost half of these genes were differentially expressed in lean vs. obese uteri irrespective of maternal OXT administration,

Table 1: Gene Expression Comparing Groups of Mice (lean vs. obese) and Treatment (± OXT) With Significant Interactions.

Gene	Lean		Obese				
	-OXT	+OXT	-OXT	+OXT			
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	p-value for interaction[a]	Main Effect Comparisons[b]	p-value
Edn1	0.992 (0.882, 1.115)	0.575 (0.516, 0.641)	0.599 (0.537, 0.667)	0.516 (0.463, 0.575)	**0.0012**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**<.0001** 0.0569 **<.0001**
Gucy1a3	0.990 (0.852, 1.151)	0.493 (0.429, 0.566)	0.769 (0.669, 0.884)	0.633 (0.551, 0.728)	**0.0013**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**<.0001** 0.0523 0.0180
Gucy1b3	0.990 (0.861, 1.139)	0.522 (0.459, 0.595)	0.781 (0.686, 0.890)	0.661 (0.581, 0.753)	**0.0012**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**<.0001** 0.0725 0.0173
Kcnq1	0.986 (0.817, 1.190)	0.622 (0.522, 0.740)	0.581 (0.488, 0.692)	0.538 (0.452, 0.641)	**0.0356**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**0.0011** 0.5257 **0.0003**
Mapk1	0.998 (0.912, 1.092)	0.712 (0.655, 0.774)	0.822 (0.756, 0.893)	0.878 (0.808, 0.955)	**<.0001**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**<.0001** 0.2569 **0.0033**
Mylk	0.952 (0.730, 1.243)	0.395 (0.309, 0.506)	0.582 (0.455, 0.745)	0.465 (0.363, 0.595)	**0.0130**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**<.0001** 0.1958 **0.0100**
Mypt3	0.997 (0.897, 1.108)	0.533 (0.483, 0.588)	0.743 (0.674, 0.820)	0.547 (0.496, 0.603)	**0.0031**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**<.0001** **0.0001** **0.0003**
Npr1	0.994 (0.826, 1.198)	0.462 (0.389, 0.548)	0.629 (0.530, 0.748)	0.435 (0.366, 0.517)	**0.0281**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**<.0001** **0.0047** **0.0011**
Pgrmc1	0.994 (0.872, 1.132)	0.720 (0.638, 0.812)	0.868 (0.769, 0.980)	0.855 (0.758, 0.965)	**0.0169**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**0.0011** 0.8576 0.1296
Pgrmc2	0.985 (0.792, 1.224)	0.667 (0.545, 0.816)	0.909 (0.743, 1.112)	1.079 (0.882, 1.320)	**0.0097**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**0.0124** 0.2260 0.5820
Prkca	0.988 (0.856, 1.140)	0.671 (0.587, 0.766)	0.736 (0.644, 0.840	0.684 (0.599, 0.782)	**0.0251**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**0.0005** 0.4358 **0.0049**

[a] $p<0.05$ is significant
[b] For these pre-specified comparisons, a Bonferroni adjustment was used such that $p<0.0167$ was considered significant.

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Table 2: Gene Expression Comparing Groups of Mice (lean vs. obese) and Treatment (± OXT) With Significant Interactions.

Gene	Lean		Obese			
	-OXT	+OXT	-OXT	+OXT	Main Effect	
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Comparisons[a]	p-value
Adipoq	0.556 (0.209, 1.484)	0.167 (0.067, 0.414)	0.683 (0.275, 1.693)	0.203 (0.082, 0.504)	Obese vs Lean No Oxt vs Oxt	0.6515 **0.0110**
Avp	0.706 (0.296, 1.687)	1.958 (0.874, 4.385)	1.420 (0.594, 3.393)	2.707 (1.208, 6.062)	Obese vs Lean No Oxt vs Oxt	0.2228 **0.0476**
Avpr	0.941 (0.706, 1.253)	1.156 (0.887, 1.507)	0.688 (0.527, 0.896)	0.627 (0.481, 0.817)	Obese vs Lean No Oxt vs Oxt	**0.0016** 0.7015
Dbn1	0.994 (0.872, 1.133)	1.418 (1.256, 1.602)	0.838 (0.742, 0.946)	1.145 (1.014, 1.293)	Obese vs Lean No Oxt vs Oxt	**0.0031** **<.0001**
Ghrl	0.982 (0.804, 1.200)	1.545 (1.283, 1.861)	0.846 (0.703, 1.019)	1.268 (1.053, 1.527	Obese vs Lean No Oxt vs Oxt	0.0638 **<.0001**
Lep	0.571 (0.218, 1.493)	0.396 (0.163, 0.965)	1.513 (0.621, 3.687)	1.096 (0.419, 2.867)	Obese vs Lean No Oxt vs Oxt	**0.0323** 0.4391
Nampt	0.989 (0.763, 1.283)	0.676 (0.531, 0.860)	1.283 (1.009, 1.632)	0.865 (0.680, 1.100)	Obese vs Lean No Oxt vs Oxt	**0.0392** **0.0027**
Nppa	0.994 (0.860, 1.150)	0.797 (0.697, 0.912)	0.890 (0.778, 1.019)	0.717 (0.627, 0.821)	Obese vs Lean No Oxt vs Oxt	0.1094 **0.0026**
Oxt	0.934 (0.512, 1.703)	1.551 (0.889, 2.705)	2.373 (1.361, 4.138)	2.164 (1.241, 3.774)	Obese vs Lean No Oxt vs Oxt	**0.0335** 0.4840
Oxtr	0.909 (0.683, 1.210)	2.994 (2.299, 3.900)	0.570 (0.438, 0.743)	1.254 (0.963, 1.634)	Obese vs Lean No Oxt vs Oxt	**<.0001** **<.0001**
Pdgfra	0.986 (0.860, 1.131)	0.956 (0.842, 1.085)	0.867 (0.764, 0.984)	0.792 (0.698, 0.899)	Obese vs Lean No Oxt vs Oxt	**0.0157** 0.3245
Plcb1	0.975 (0.816, 1.165)	1.645 (1.395, 1.940)	0.798 (0.677, 0.941)	1.516 (1.285, 1.788)	Obese vs Lean No Oxt vs Oxt	0.0980 **<.0001**
Prkcab	0.990 (0.891, 1.100)	0.716 (0.650, 0.790)	0.811 (0.736, 0.894)	0.707 (0.642, 0.780)	Obese vs Lean No Oxt vs Oxt	0.0553 **0.0002**
Prkcg	0.994 (0.810, 1.219)	1.152 (0.954, 1.392)	0.615 (0.509, 0.743)	0.783 (0.648, 0.946)	Obese vs Lean No Oxt vs Oxt	**<.0001** **0.0426**
Ptgs1	0.989 (0.829, 1.181)	0.587 (0.499, 0.692)	0.670 (0.569, 0.789)	0.539 (0.457, 0.635)	Obese vs Lean No Oxt vs Oxt	**0.0116** **0.0003**

[a]For these transcripts the interaction between group/BMI and OXT treatment (yes,no) was not significant ($p \geq 0.05$) so the interaction was removed and the main effects of group and treatment were examined. Therefore, $p<0.05$ was considered significant.

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with higher expression in lean vs. obese uteri for *Avpr, Dbn1, Oxtr, Pdgfra, Prkcg, and Ptgs1* or lower expression in lean vs. obese uteri for *Lep, Nampt,* and *Oxt* (Table 2). More than half of the genes examined showed significant responsiveness to maternal OXT treatment irrespective of dam weights, including *Adipoq, Avp, Dbn1, Ghrl, Nampt, Nppa, Oxtr, Plcb1, Prkacb, Prkcg,* and *Ptgs1 (Table 2).* Specifically, *Avp, Dbn1, Ghrl, Oxtr, Plcb1, and Prkcg* were increased following maternal OXT administration, *while Adipoq, Nampt, Nppa, Prkacb,* and *Ptgs1* were decreased (Table 2).

OXT Dosing in Obese Dams Results in Blunted Uterine Gene Expression Responses

Next, we examined the differences in the expression of uterine genes following vehicle (no OXT) vs. reg-dose OXT vs. hi-dose OXT administered to obese dams at term. The full dataset is in Supplementary Table 3. Among the same 30 genes analyzed in experiment 1, this dose escalation study revealed that 9 genes (*Dbn1, Ghrl, Gucy1a3, Mypt3, Npr1, Oxtr, Plcb1, Prkcg, and Ptgs2*) showed an overall significant dose effect (vs. vehicle) (Figure 1). Although *Npr1* showed an overall significant p value ($p<0.05$) across all doses (vehicle, reg-dose, and hi-dose), no pairwise comparisons were significant (Supplementary Table 3) and thus it was not included in Figure 1. Among the 8 remaining genes, all increased following maternal OXT, except for *Mypt3* and *Gucy1a3*, which decreased with OXT (Figure 1). While 7 genes showed significant differential expression for pairwise comparisons of hi-dose OXT vs. no OXT (vehicle) in obese mice, most exhibited a 'plateau effect' with increasing OXT doses in obese mice (i.e. a 'blunted OXT response') (Figure 1). Only *Ptgs2* showed a significant difference for the most clinically relevant comparison, reg-dose OXT vs. hi-dose OXT ($p=0.0083$) (Figure 1).

Discussion

Rising rates of obesity among women of reproductive age are concerning [24] and there is ample evidence that obesity negatively impacts parturition, resulting in delayed labor, higher rates of labor dystocia, and poor outcomes [6-10, 14, 16]. The high prevalence of obesity may contribute to the high percentage of pregnant women receiving OXT to facilitate labor in the US [2]. While several baseline differences in lean vs. obese uteri/myometrium have been described [25] and some limited gene and protein expression profiling of the myometrium in pregnancy and in labor have been performed [26], the effects of obesity on myometrial maturation and labor induction and progression are incompletely understood.

Using a 2x2 study design to compare lean (±OXT) and obese (±OXT) groups of mice for gene expression involved in uterine contraction and relaxation, as well as labor and obesity (e.g. genes related to adipokines, gap junctions, and inflammation) allowed assessment of an interaction between obesity and OXT-relating signaling during parturition. Nine genes showed significant interactions, including *Edn1, Gucy1a3, Gucy1b3, Kcnq1, Mapk1, Mylk, Pgrmc1, Pgrmc2,* and *Prkca*, with OXT responsiveness only in lean mice (Table 1) – supporting that the OXT effect depended on body mass. Only *Npr1* and *Mypt3* (whose gene products play roles in the relaxation pathway) were significantly downregulated in both lean and obese mice by OXT, with greater effects in lean mice (Table 1). These data identify several myometrial mRNA transcripts implicated in reduced OXT responsiveness in obese mice (Figure 2).

For the remaining genes that showed no significant interactions (i.e. the effect of OXT did not depend on the dams' body mass), the main effects of group (lean vs. obese) and treatment (no OXT vs. OXT) identified several genes implicated in parturition that were differentially expressed in obese and lean uteri (Table 2 and Figure 2). The expression of uterine contractility-related genes that encode OXTR (*Oxtr*) and phospholipase C beta (*Plcb1*), a downstream effector of G-protein-coupled receptor signaling that has central roles in labor, were significantly higher in lean uteri and both genes were significantly upregulated in lean and obese dams following maternal OXT ($p<0.0001$). To our knowledge and based on a review of the literature, *Plcb1* expression has not been assessed in lean vs. obese uteri. Interestingly, maternal obesity does not appear to influence myometrial OXTR gene or protein expression in humans [27]. This discrepancy may be due to differences in sampling sites, timing, or species. Clearly, assessment of myometrial OXTR protein expression and functional assessment of contractility ex vivo are warranted as hypercholesterolemia is associated with labor dysfunction and impaired OXT responsiveness [28, 29]. Cholesterol treatment of isolated uterine smooth muscle cells reduces contractility [30], possibly by reducing membrane fluidity and impairing OXTR function, as previously described for other membrane receptors [31].

Previous studies using a similar HFD model in rats reported decreased Cx43 (encoded by *Gja1*) protein expression in the pregnant uteri of obese vs. lean rats [32]. Cx43 is a major myometrial gap junction protein proposed to synchronize myometrial contractions [33]. In our study, *Gja1* (encodes Cx43) was not differentially expressed in lean vs. obese mice (Supplementary Table 2). This difference may be related to the difference in species and/or mRNA vs. protein assessment. Interestingly, we found that *Dbn1* (that encodes a Cx43 binding partner) was differentially expressed in lean and obese myometrium, with higher expression in lean myometrium (Table 2). While *Dbn1* is upregulated in the pregnant myometrium [34], little is known about its function and to our knowledge its expression has not been linked to obesity.

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A panel of adipokine genes was also examined based on their proposed contribution to labor dysfunction and reports that adipokines inhibit uterine contractility [35-39]. Originally thought to be expressed only by adipocytes, adipokine mRNAs and proteins are expressed by the myometrium (e.g. *Lep*/leptin [40, 41], *Adipoq*/adiponectin [42, 43], *Nampt*/visfatin [44], and *Ghrl*/ghrelin [45]). Not all studies report similar findings; these inconsistencies may be due to detection sensitivity, species differences, and/or tissue state (pregnant, non-pregnant, healthy, and pathologic). Consistent with obesity-induced leptin expression [46]. *Lep* was more highly expressed in obese uteri; however, it was unchanged by OXT (Supplementary Table 2). By contrast, OXT altered *Adipoq, Ghrl* and *Nampt* mRNA expression in both lean and obese uteri (Table 2). While these data suggest that OXT may not exert differential effects in obese uteri through these adipokine mRNAs, adipokine receptor expression should be examined.

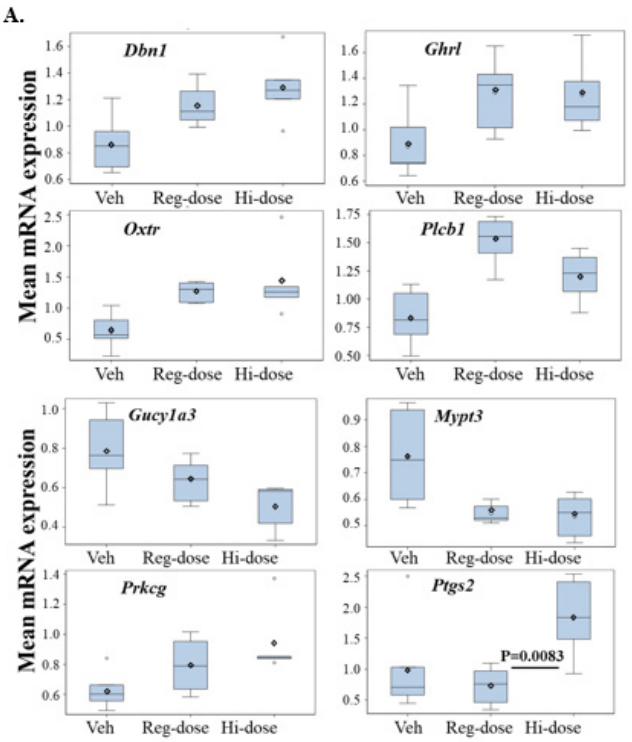
Clinically, since obese patients may require higher OXT doses to augment labor [4, 11-13], we performed a dose escalation study using the same obesity model and gene panel comparing hi- vs. reg-dose OXT. Only one gene (among 30 examined), *Ptgs2*, showed a significant difference when comparing the clinically relevant comparison of hi- vs. reg-OXT doses (Figure 1, lower panel). Previous studies report that OXT upregulates *Ptgs2* gene and COX-2 protein expression using cultured endometrial epithelial cells, supporting its role in mediating OXT-induced PGF2α production by these cells [47]. In addition, COX-2 expression is increased during labor [48]. The major differences between our study and prior studies were the use of an in vivo model and analysis of myometrial tissues vs. cultured epithelial cells. Interestingly, COX-2 inhibition has been proposed to mediate myometrial relaxation in vitro [49]. Overall, the results of the dose escalation study support that obesity reduces uterine OXT-responsiveness and suggest that future research focusing on enhancing OXTR responsiveness in the setting of obesity may be informative.

This study has several limitations. First, we only assessed uterine gene expression at one time point (6 hours post-OXT) and therefore, we lack a timeline of gene expression changes. Second, the reg-dose of OXT was relatively high; however, it was based on previous experimental work investigating the effects of OXT on parturition [21]. In our preliminary experiments, this dose did not induce labor and we avoided delayed labor reported with lower OXT doses OXT [21]. In addition, we did not assess global gene expression. Nor did we measure protein products or evaluate functional myometrial contractility. However, results from prior studies support that obesity/hypercholesterolemia (a feature of this obesity model) is associated with labor dysfunction, altered uterine contractility and OXT responsiveness [28, 29, 32] and together with our data, support future studies using a similar model. Finally, there are inherent limitations in translating findings from pregnant mice to pregnant humans.

Conclusions

In conclusion, lean and obese uteri exhibit significantly different gene expression profiles and obese uteri show blunted gene expression effects reflecting reduced baseline differences and OXT responsiveness (Figure 2). These

A.



B.

	p-values for Pairwise Comparisons*			
Gene	**Overall p-value**	**Hi-dose Oxt vs -Oxt**	**Reg-dose Oxt vs. -Oxt**	**Hi-dose Oxt vs reg-dose Oxt**
Oxtr	0.0006	0.0007	0.0007	0.7338
Ghrl	0.0072	0.0100	0.0041	0.8950
Ptgs2	0.0220	0.0319	0.4808	**0.0083**
Mypt3	0.0018	0.0018	0.0018	0.7351
Gucy1a3	0.0086	0.0024	0.1038	0.0592
Dbn1	0.0026	0.0013	0.0057	0.3446
Prkcg	0.0103	0.0034	0.0402	0.1821
Plcb1	0.0002	0.0067	<0.0001	0.0633

Figure 1: Variable Effects of Increasing OXT Doses on Uterine Gene Expression in Obese Dams.

Obese dams were treated with either vehicle (Veh), regular dose OXT (1U/100µl every 30min for 2h, reg-dose) or high dose (2U/100µl every 30min for 2h, hi-dose). (A) A significant interaction was found for *Dbn1, Ghrl, Mypt3, Gucy1a3, Oxtr, Plcb1, Prkcg,* and *Ptgs2* genes, which were then analyzed using pairwise comparisons using ANOVA model. Box plots in the above panels describe the distribution of gene expression data in uteri from obese mice following treatment saline, reg-dose, or hi-dose OXT. ◊ = mean; center line = median; top and bottom of boxes = 75%-ile and 25%-ile, respectively; whiskers extend to minimum and maximum with outliers shown separately. (B) P values for the overall and pairwise comparisons are shown in the table; * for pairwise comparisons, a Bonferroni adjustment was used, such that $p<0.0167$ was considered significant.

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Obesity significantly reduces the expression of several parturition-related genes in the myometrium when compared to lean dams at term

Contraction-related genes			Relaxation-related genes	
Avpr	*Dbn1*	*Edn1*	*Kcnq1*	*Npr1*
Mapk1	*Mylk*	*Mypt3*		
Oxtr	*Pdgfra*	*Prkca*		
Prkcg	*Ptgs1*			

OXT-induced gene expression changes observed in lean myometrium at term are blunted or not observed in obese myometrium at term

Contraction-related genes		Relaxation-related genes	
Edn1	Δ by OXT, lean only	*Gucy1a3*	Δ by OXT, lean only
Mapk1	Δ by OXT, lean only	*Gucy1b3*	Δ by OXT, lean only
Mylk	Δ by OXT, lean only	*Kcnq1*	Δ by OXT, lean only
Pgrmc1	Δ by OXT, lean only	*Mypt3*	Δ by OXT, lean > obese
Pgrmc2	Δ by OXT, lean only	*Npr1*	Δ by OXT, lean > obese
Prkca	Δ by OXT, lean only		

Figure 2: Differential Expression of Parturition-Related Myometrium Genes in Setting of Obesity.
Several contraction- and relaxation-related genes show differential expression in the myometrium of lean vs. obese pregnant mice, with significantly reduced gene expression in obese vs. lean mice (upper panel). In addition, several contraction- and relaxation-related genes in the myometrium exhibit decreased OXT responsiveness in obesity pregnant mice when compared to lean pregnant mice (lower panel). Δ = change.

effects may contribute to uncoordinated contractions and obesity-associated labor dystocia. For providing optimal delivery experiences with fewer adverse outcomes, future studies should focus on 1) defining body mass-based OXT dosing regimens through pre-clinical (animal) and clinical dosing studies and 2) investigating ways to improve OXT responsiveness and regulate uterine contractility and relaxation, using functional studies.

Declarations

Ethics Approval and Consent to Participate/Consent for Publication

The Institutional Animal Care and Use Committee (IACUC) of the Feinstein Institutes for Medical Research reviewed and approved the animal studies (IACUC #2015-053) prior to animal experimentation. All animal experimentation was in accordance with the guidelines for animal care provided by the National Institutes of Health. No human experimentation was performed. No human subjects were involved in this research and therefore, there was no consent process.

Availability of Data and Materials

All data generated and analyzed during this study are included in this published manuscript (or included in the Supplemental Data).

Competing Interests, Conflict of Interest

All authors confirmed that all methods were performed in accordance with the relevant guidelines and regulations in research. The authors declare that they have no competing interests or conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

Authors' Contributions

SS, FFH, BR and CNM conceived experiments and designed the study. SS, FFH, XX, and CNM performed animal experimentation, sample collections and sample processing. SS, FFH and PKC performed RNA isolation and assessment of RNA quality. SS and PKC performed qPCR; SS, PKC, SM and CNM contributed to data interpretation and preparation of Tables and Figures. SS, SM, and CNM wrote the manuscript with SS and SM. NK performed all data analyses and reviewed the results with the authors. All authors reviewed, edited. and approved the final manuscript, Tables and Figures, as well as Supplementary data. SS is currently at the Center for Fetal Diagnosis and Treatment, Children's Hospital of Philadelphia, 34th Street and Civic Center Boulevard, 5 Wood, Philadelphia, PA 19104 USA FFH is currently at Stamford Hospital, One Hospital Plaza, Stamford, CT 06905 USA

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SUPPLEMENTARY DATA

Supplementary Table 1: List of genes (and encoded proteins) associated with parturition, OXT signaling, and obesity with primers for real time-qPCR.

Gene		Primers	Encodes
Adipoq	Forward	AGGGAGAGAAAGGAGATGCAG	Adiponectin
	Reverse	CTTTCCTGCCAGGGGTTC	
Avp	Forward	CTACGCTCTCCGCTTGTTTC	Arginine vasopressin
	Reverse	GGGCAGTTCTGGAAGTAGCA	
Avpr1	Forward	GGGATACCAATTTCGTTTGG	Arginine vasopressin receptor 1A
	Reverse	AAGCCAGTAACGCCGTGAT	
Dbn1	Forward	TAACCCACGGGAGTTCTTCA	Debrin 1
	Reverse	AAAGGGCAGTACGGACGAC	
Edn1	Forward	TCCTTGATGGACAAGGAGTGT	Endothelin 1
	Reverse	CCCAGTCCATACGGTACGA	
Gapdh	Forward	GAGCCAAACGGGTCATCA	Glyceraldehyde 3-phosphate dehydrogenase
	Reverse	CATATTTCTCGTGGTTCACACC	
Ghrl	Forward	CCAGAGGACAGAGGACAAGC	Ghrelin
	Reverse	ACATCGAAGGGAGCATTGAA	
Gja1	Forward	TTTGACTTCAGCCTCCAAGG	Connexin 43
	Reverse	CATGTCTGGGCACCTCTCTT	
Gucy1a3	Forward	TACACTCGCTTTGACCAGCA	Guanylate cyclase soluble subunit α3
	Reverse	AATATGCATCCCCGATGGT	
Gucy1b3	Forward	CGTCTCAAAGGCCAAATGAT	Guanylate cyclase soluble subunit β3
	Reverse	TCGTCCAGGTTCATCACACT	
Kcnq1	Forward	ACTGCTGACCCCCATCAC	Potassium voltage-gated channel Q member 1
	Reverse	CATGCGCCTGATGACCTT	
Lep	Forward	CAGGATCAATGACATTTCACACA	Leptin
	Reverse	GCTGGTGAGGACCTGTTGAT	
Lpar1	Forward	GCCTCTACTTCCAGCCCTGT	Lysophosphatidic acid receptor 1
	Reverse	GCACTGTTGTTCGTTCATGG	
Mapk1	Forward	AAGAACTCATTTTTGAAGAGACTGC	MAP Kinase 1
	Reverse	CTCTGAGCCCTTGTCCTGA	
Mylk	Forward	GCCAGGTCACTATGACAGTCC	Myosin light chain kinase
	Reverse	CGTCGTGAAGCCAGATGAC	
Mypt3	Forward	CCTCGGAAGCATGTCCTCT	Myosin phosphatase target subunit 3
	Reverse	GGTAAGGAACTGGCGGACT	
Nampt	Forward	TGTTCCAGGCTATTCTGTTCC	Visfatin
	Reverse	TTCAAAAGCATCTTTCTCATGG	
Nppa	Forward	CAACACAGATCTGATGGATTTCA	Natriuretic peptide A
	Reverse	CCTCATCTTCTACCGGCATC	
Npr1	Forward	TGGAGACACAGTCAACACAGC	Natriuretic peptide receptor 1
	Reverse	CGAAGACAAGTGGATCCTGAG	
Oxtr	Forward	AGCGTCTGGGACGTCAAT	Oxytocin receptor
	Reverse	GTTGAGGCTGGCCAAGAG	
Oxt	Forward	CACCTACAGCGGATCTCAGAC	Oxytocin
	Reverse	CGAGGTCAGAGCCAGTAAGC	
Pdgfra	Forward	AAGACCTGGGCAAGAGGAAC	Platelet-derived growth factor receptor alpha
	Reverse	GAACCTGTCTCGATGGCACT	

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Pgr	Forward	TGCACCTGATCTAATCCTAAATGA	Progesterone receptor (PGR)
	Reverse	GGTAAGGCACAGCGAGTAGAA	
Pgrmc1	Forward	CACAGCAGGAGACCCTGAGT	PGR membrane component 1
	Reverse	CTCCCCTTCCTTCAGCAGTT	
Pgrmc2	Forward	TGTTCGAGAATGGGAAATGC	PGR membrane component 2
	Reverse	GATCCTTGGTGTCCTCCTCA	
Plcb1	Forward	TCGATGAGAAGCCCAAGC	Phospholipase C beta 1
	Reverse	GGCAGCCTTTTGAACTTGTC	
Prkcab	Forward	TCAAGCCGGAAAACCTCTTA	Protein kinase cAMP-activated catalytic beta
	Reverse	CTTGACTCTTTTGGCGAACC	
Prkca	Forward	CAAGGGATGAAATGTGACACC	Protein kinase C alpha
	Reverse	CCTCTTCTCTGTGTGATCCATTC	
Prkcg	Forward	GTTTGAGGCCTGCAATTACC	Protein kinase C gamma
	Reverse	AGAGTTCGTCGGAGCCTCT	
Ptgs1	Forward	CCTCTTTCCAGGAGCTCACA	Cyclooxygenase (COX)-1
	Reverse	TCGATGTCACCGTACAGCTC	
Ptgs2	Forward	GGGAGTCTGGAACATTGTGAA	Cyclooxygenase (COX)-2
	Reverse	TGTCAATCAAATATGATCTGGATGT	

Supplementary Table 2: All data for experiment 1: Gene expression comparing groups (lean vs. obese) and treatment (± OXT).

Gene	Lean		Obese				
	-OXT	+OXT	-OXT	+OXT		Main Effect	
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	p-value for inter-action[a]	Comparisons[b]	p-value
Adipoq	0.556 (0.209, 1.484)	0.167 (0.067, 0.414)	0.683 (0.275, 1.693)	0.203 (0.082, 0.504)	NS	Obese vs Lean No Oxt vs Oxt	0.6515 **0.0110**
Avp	0.706 (0.296, 1.687)	1.958 (0.874, 4.385)	1.420 (0.594, 3.393)	2.707 (1.208, 6.062)	NS	Obese vs Lean No Oxt vs Oxt	0.2228 **0.0476**
Avpr	0.941 (0.706, 1.253)	1.156 (0.887, 1.507)	0.688 (0.527, 0.896)	0.627 (0.481, 0.817)	NS	Obese vs Lean No Oxt vs Oxt	**0.0016** 0.7015
Dbn1	0.994 (0.872, 1.133)	1.418 (1.256, 1.602)	0.838 (0.742, 0.946)	1.145 (1.014, 1.293)	NS	Obese vs Lean No Oxt vs Oxt	**0.0031** **<.0001**
Edn1	0.992 (0.882, 1.115)	0.575 (0.516, 0.641)	0.599 (0.537, 0.667)	0.516 (0.463, 0.575)	**0.0012**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**<.0001** 0.0569 **<.0001**
Ghrl	0.982 (0.804, 1.200)	1.545 (1.283, 1.861)	0.846 (0.703, 1.019)	1.268 (1.053, 1.527	NS	Obese vs Lean No Oxt vs Oxt	0.0638 **<.0001**
Gja1	0.978 (0.769, 1.244)	1.098 (0.879, 1.371)	1.059 (0.848, 1.323)	1.081 (0.865, 1.351)	NS	Obese vs Lean No Oxt vs Oxt	0.7807 0.5443
Gucy1a3	0.990 (0.852, 1.151)	0.493 (0.429, 0.566)	0.769 (0.669, 0.884)	0.633 (0.551, 0.728)	**0.0013**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**<.0001** 0.0523 0.0180
Gucy1b3	0.990 (0.861, 1.139)	0.522 (0.459, 0.595)	0.781 (0.686, 0.890)	0.661 (0.581, 0.753)	**0.0012**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**<.0001** 0.0725 0.0173

Citation: Shelly Soni, Prodyot K Chatterjee, Frances F Hsieh, Xiangying Xue, Nina Kohn, Swati Madankumar, Burton Rochelson and Christine N Metz. Altered Uterine Gene Expression in Lean and Obese Mice Following Maternal Oxytocin. Journal of Women's Health and Development 5 (2022): 206-220.

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Kcnq1	0.986 (0.817, 1.190)	0.622 (0.522, 0.740)	0.581 (0.488, 0.692)	0.538 (0.452, 0.641)	**0.0356**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**0.0011** 0.5257 **0.0003**
Lep	0.571 (0.218, 1.493)	0.396 (0.163, 0.965)	1.513 (0.621, 3.687)	1.096 (0.419, 2.867)	NS	Obese vs Lean No Oxt vs Oxt	**0.0323** 0.4391
Lpar1	0.984 (0.852, 1.138)	0.911 (0.797, 1.042)	0.922 (0.806, 1.054)	1.034 (0.904, 1.182)	NS	Obese vs Lean No Oxt vs Oxt	0.6149 0.7411
Mapk1	0.998 (0.912, 1.092)	0.712 (0.655, 0.774)	0.822 (0.756, 0.893)	0.878 (0.808, 0.955)	**<.0001**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**<.0001** 0.2569 **0.0033**
Mylk	0.952 (0.730, 1.243)	0.395 (0.309, 0.506)	0.582 (0.455, 0.745)	0.465 (0.363, 0.595)	**0.0130**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**<.0001** 0.1958 **0.0100**
Mypt3	0.997 (0.897, 1.108)	0.533 (0.483, 0.588)	0.743 (0.674, 0.820)	0.547 (0.496, 0.603)	**0.0031**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**<.0001** **0.0001** **0.0003**
Nampt	0.989 (0.763,1.283)	0.676 (0.531,0.860)	1.283 (1.009,1.632)	0.865 (0.680,1.100)	NS	Obese vs Lean No Oxt vs Oxt	**0.0392** **0.0027**
Nppa	0.994 (0.860, 1.150)	0.797 (0.697, 0.912)	0.890 (0.778, 1.019)	0.717 (0.627, 0.821)	NS	Obese vs Lean No Oxt vs Oxt	0.1094 **0.0026**
Npr1	0.994 (0.826, 1.198)	0.462 (0.389, 0.548)	0.629 (0.530, 0.748)	0.435 (0.366, 0.517)	**0.0281**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**<.0001** **0.0047** **0.0011**
Oxt	0.934 (0.512, 1.703)	1.551 (0.889, 2.705)	2.373 (1.361, 4.138)	2.164 (1.241, 3.774)	NS	Obese vs Lean No Oxt vs Oxt	**0.0335** 0.4840
Oxtr	0.909 (0.683, 1.210)	2.994 (2.299, 3.900)	0.570 (0.438, 0.743)	1.254 (0.963, 1.634)	NS	Obese vs Lean No Oxt vs Oxt	**<.0001** **<.0001**
Pdgfra	0.986 (0.860, 1.131)	0.956 (0.842, 1.085)	0.867 (0.764, 0.984)	0.792 (0.698, 0.899)	NS	Obese vs Lean No Oxt vs Oxt	**0.0157** 0.3245
Pgr	0.983 (0.816, 1.184)	0.894 (0.753, 1.062)	0.792 (0.667, 0.940)	0.759 (0.639, 0.902)	NS	Obese vs Lean No Oxt vs Oxt	**0.0326** 0.4272
Pgrmc1	0.994 (0.872, 1.132)	0.720 (0.638, 0.812)	0.868 (0.769, 0.980)	0.855 (0.758, 0.965)	**0.0169**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**0.0011** 0.8576 0.1296
Pgrmc2	0.985 (0.792, 1.224)	0.667 (0.545, 0.816)	0.909 (0.743, 1.112)	1.079 (0.882, 1.320)	**0.0097**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**0.0124** 0.2260 0.5820
Plcb1	0.975 (0.816, 1.165)	1.645 (1.395, 1.940)	0.798 (0.677, 0.941)	1.516 (1.285, 1.788)	NS	Obese vs Lean No Oxt vs Oxt	0.0980 **<.0001**
Prkacb	0.990 (0.891, 1.100)	0.716 (0.650, 0.790)	0.811 (0.736, 0.894)	0.707 (0.642, 0.780)	NS	Obese vs Lean No Oxt vs Oxt	0.0553 **0.0002**
Prkca	0.988 (0.856, 1.140)	0.671 (0.587, 0.766)	0.736 (0.644, 0.840	0.684 (0.599, 0.782)	**0.0251**	Lean: Oxt vs No Oxt Obese: Oxt vs No Oxt No Oxt: Obese vs Lean	**0.0005** 0.4358 **0.0049**
Prkcg	0.994 (0.810, 1.219)	1.152 (0.954, 1.392)	0.615 (0.509, 0.743)	0.783 (0.648, 0.946)	NS	Obese vs Lean No Oxt vs Oxt	**<.0001** **0.0426**

Citation: Shelly Soni, Prodyot K Chatterjee, Frances F Hsieh, Xiangying Xue, Nina Kohn, Swati Madankumar, Burton Rochelson and Christine N Metz. Altered Uterine Gene Expression in Lean and Obese Mice Following Maternal Oxytocin. Journal of Women's Health and Development 5 (2022): 206-220.

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Ptgs2	0.930 (0.633, 1.367)	0.687 (0.481, 0.981)	0.830 (0.565, 1.220)	0.672 (0.457, 0.987)	NS	Obese vs Lean No Oxt vs Oxt	0.7118 0.1584
Ptgs1	0.989 (0.829, 1.181)	0.587 (0.499, 0.692)	0.670 (0.569, 0.789)	0.539 (0.457, 0.635)	NS	Obese vs Lean No Oxt vs Oxt	**0.0116** **0.0003**

[a]p<0.05 is significant
[b]Where interaction between group/BMI and OXT treatment (yes,no) was significant (p<0.05), pre-specified comparisons were examined within the ANOVA model. For these comparisons, a Bonferroni adjustment was used, such that p<0.0167 was considered significant. Where the interaction between group and OXT was not significant (NS), the interaction was removed and only the main effects of group and treatment (OXT) were examined.

Supplementary Table 3: All data for experiment 2: Gene expression comparing vehicle vs. regular- and high-dose OXT in obese uteri.

	Veh (-OXT)	**Reg-dose[a]**	**Hi-dose[b]**			
Gene	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	**p-value for effect of OXT[c]**	**Comparison[d]**	**p-value**
Adipoq	0.683 (0.317,1.472)	0.203 (0.094,0.438)	0.252 (0.101, 0.624)	0.0723		
Avp	1.420 (0.632, 3.191)	2.707 (1.279, 5.727)	1.908 (0.786, 4.632)	0.4753		
Avpr	0.688 (0.524, 0.902)	0.627 (0.478, 0.823)	0.716 (0.519, 0.987)	0.7828		
Dbn1	0.838 (0.724, 0.971)	1.145 (0.989, 1.326)	1.271 (1.068, 1.512)	**0.0026**	Veh vs Hi	**0.0013**
					Reg vs Hi	0.3446
					Reg vs Veh	**0.0057**
Edn1	0.599 (0.522, 0.687)	0.516 (0.450, 0.592)	0.543 (0.462, 0.639)	0.2874		
Ghrl	0.846 (0.706, 1.014)	1.268 (1.058, 1.520)	1.246 (1.005, 1.544)	**0.0072**	Veh vs Hi	**0.0100**
					Reg vs Hi	0.8950
					Reg vs Veh	**0.0041**
Gja1	1.059 (0.807, 1.389)	1.081 (0.824, 1.418)	1.503 (1.091, 2.072)	0.1870		
Gucy1a3	0.769 (0.649, 0.911)	0.633 (0.534, 0.750)	0.492 (0.403, 0.601)	**0.0086**	Veh vs Hi	**0.0024**
					Reg vs Hi	0.0592
					Reg vs Veh	0.1038
Gucy1b3	0.781 (0.681, 0.897)	0.661 (0.576, 0.759)	0.618 (0.525, 0.727)	0.0745		
Kcnq1	0.581 (0.467, 0.723)	0.538 (0.433, 0.670)	0.445 (0.344, 0.576)	0.2674		
Lep	1.513 (0.885, 2.587)	1.096 (0.614, 1.955)	0.961 (0.509, 1.812)	0.4852		
Lpar1	0.922 (0.795, 1.069)	1.034 (0.892, 1.199)	1.071 (0.899, 1.276)	0.3439		
Mapk1	0.822 (0.759, 0.890)	0.878 (0.811, 0.951)	0.827 (0.753, 0.909)	0.4228		
Mylk	0.582 (0.441, 0.768)	0.465 (0.352, 0.614)	0.428 (0.308, 0.595)	0.2963		

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Mypt3	0.743 (0.657, 0.841)	0.547 (0.483, 0.619)	0.530 (0.458, 0.613)	**0.0018**	Veh vs Hi	**0.0018**
					Reg vs Hi	0.7351
					Reg vs Veh	**0.0018**
Nampt	1.283 (0.963, 1.709)	0.865 (0.650, 1.152)	1.076 (0.766, 1.510)	0.1511		
Nppa	0.890 (0.758, 1.046)	0.717 (0.611, 0.842)	0.761 (0.629, 0.921)	0.1502		
Npr1	0.629 (0.501, 0.791)	0.435 (0.346, 0.547)	0.412 (0.314, 0.540)	**0.0339**	Veh vs Hi	0.0221
					Reg vs Hi	0.7446
					Reg vs Veh	0.0281
Oxt	2.373 (1.305, 4.313)	2.164 (1.191, 3.934)	1.707 (0.842, 3.462)	0.7503		
Oxtr	0.570 (0.431, 0.755)	1.254 (0.948, 1.660)	1.346 (0.966, 1.876)	**0.0006**	Veh vs Hi	**0.0007**
					Reg vs Hi	0.7338
					Reg vs Veh	**0.0007**
Pdgfra	0.867 (0.752, 1.000)	0.792 (0.687, 0.913)	0.878 (0.742, 1.039)	0.5343		
Pgr	0.792 (0.661, 0.949)	0.759 (0.634, 0.910)	0.706 (0.570, 0.875)	0.6914		
Pgrmc1	0.868 (0.752, 1.002)	0.855 (0.740, 0.988)	0.949 (0.800, 1.125)	0.5897		
Pgrmc2	0.909 (0.710, 1.164)	1.079 (0.842, 1.382)	1.127 (0.841, 1.511)	0.4410		
Plcb1	0.798 (0.672, 0.948)	1.516 (1.277, 1.800)	1.180 (0.963, 1.446)	**0.0002**	Veh vs Hi	**0.0067**
					Reg vs Hi	0.0633
					Reg vs Veh	**<0.0001**
Prkacb	0.811 (0.740, 0.888)	0.707 (0.646, 0.775)	0.731 (0.656, 0.814)	0.0974		
Prkca	0.736 (0.627, 0.863)	0.684 (0.584, 0.803)	0.600 (0.497, 0.725)	0.2462		
Prkcg	0.615 (0.523, 0.723)	0.783 (0.666, 0.921)	0.924 (0.763, 1.120)	**0.0103**	Veh vs Hi	**0.0034**
					Reg vs Hi	0.1821
					Reg vs Veh	0.0402
Ptgs1	0.670 (0.531, 0.845)	0.539 (0.427, 0.679)	0.560 (0.426, 0.737)	0.3563		
Ptgs2	0.830 (0.533, 1.295)	0.672 (0.431, 1.047)	1.726 (1.061, 2.807)	**0.0220**	Veh vs Hi	0.0319
					Reg vs Hi	**0.0083**
					Reg vs Veh	0.4808

[a]Hi-dose=high-dose OXT
[b]Reg-dose=regular dose OXT
[c]p<0.05 is significant
[d]Where there was a significant effect of OXT (p<0.05), pairwise comparisons were carried out within the ANOVA model. A Bonferroni adjustment was used, such that p<0.0167 was considered significant for these comparisons.