THE ROLE OF PROSTAGLANDINS IN DISRUPTED GASTRIC MOTOR ACTIVITY ASSOCIATED WITH TYPE 2 DIABETES

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ABSTRACT

Patients with diabetes mellitus often develop gastrointestinal motor problems, including gastroparesis. Previous studies have suggested this gastric motor disorder was a consequence of an enteric neuropathy. Disruptions in interstitial cells of Cajal (ICC) have also been reported. A thorough examination of functional changes in gastric motor activity during diabetes has not yet been performed. We comprehensively examined the gastric antrums of Lep$^{ob}$ mice using functional, morphological and molecular techniques to determine the pathophysiological consequences in this type 2 diabetic animal model. Video analysis and isometric force measurements revealed higher frequency and less robust antral contractions in Lep$^{ob}$ mice compared to controls. Electrical pacemaker activity was reduced in amplitude and increased in frequency. Populations of enteric neurons, ICC and PDGFRα$^+$ cells were unchanged. Analysis of components of the prostaglandin pathway revealed upregulation of multiple enzymes and receptors. Prostaglandin-endoperoxide synthase-2 (PTGS2) inhibition increased slow wave amplitudes and reduced frequency of diabetic antrums. In conclusion, gastric pacemaker and contractile activity is disordered in type 2 diabetic mice and this appears to be a consequence of excessive prostaglandin signaling. Inhibition of prostaglandin synthesis may provide a novel treatment for diabetic gastric motility disorders.
INTRODUCTION

Diabetes mellitus is often accompanied by gastrointestinal (GI) motility dysfunction (1). The gastric complications of diabetes are frequently attributed to an enteric neuropathy, primarily due to loss or reduced expression of neuronal nitric oxide synthase (NOS1) inhibitory neurons (2,3). Conversely, reduced populations of excitatory enteric neurons have also been reported (4). Disruption of gastric interstitial cells of Cajal (ICC) have also been reported in animal models of diabetes (5,6) and in human patients (7,8). Studies have shown that ICC are the GI pacemaker cells that generate electrical slow waves which underlie phasic contractions throughout the GI tract (9–11). These cells also act as intermediaries in enteric neuroeffector transmission (12,13). Thus, defects or loss of ICC may be involved in the pathological motor activities in gastroparesis. Aberrant electrical activity (5), development of delayed gastric emptying and loss of ICC have been observed concomitantly in animal models of diabetes (14,15). In contrast, an increase, rather than a decrease, in gastric emptying has been observed in approximately 20% of diabetic patients (16). Recently, an increase in gastric ICC numbers and increased cholinergic motor responses have been detected in female Lepr\textsuperscript{db} mice, and these changes were associated with accelerated gastric emptying (17). Overall, the pathogenesis of the GI motor complications of diabetes remain poorly understood, and there are a paucity of studies examining changes in ICC, enteric neurons and the functional deficits associated with type 2 diabetes.

Prostaglandins are biologically active lipid derivatives of arachidonic acid that are synthesized in GI muscles (18). Arachidonic acid is liberated from membrane phospholipids by phospholipase A\textsubscript{2} and subsequently converted to prostaglandin H\textsubscript{2} (PGH\textsubscript{2}) by the prostaglandin-endoperoxide synthase enzymes (PTGS; also known as cyclooxygenase). Two isoforms of PTGS exist: PTGS1 and PTGS2. PTGS1 is constitutively expressed whereas PTGS2 expression is
generally low under normal conditions but can be induced by inflammation (19). However, constitutive expression of PTGS2 has been observed in intramuscular ICC (ICC-IM) and enteric neurons of the GI tract (20,21). PGH2 is then converted to various prostaglandins and thromboxane (i.e. PGE2, PGD2, PGF2α, PGI2 and TXA2; see Fig. 7) by several specific prostaglandin synthase enzymes (22). For example, PGE2 is produced by microsomal prostaglandin E2 synthase (PTGES; or mPGES). Like PTGS, there are multiple isoforms of this enzyme (PTGES, PTGES2 and PTGES3), of which PTGES2 and PTGES3 are constitutively expressed and PTGES is induced by inflammatory mediators (19).

Endogenous prostaglandins (particularly prostaglandin E2; PGE2) affect gastric motility. PGE2 affects pacemaker activity in the antrum by increasing the frequency and decreasing the amplitude of slow waves (23,24). PGE2 also inhibits phasic contractions in the antrum but enhances contractile tone in the proximal stomach (20,23). Consequently, this disrupts the coordination of gastric peristalsis in intact muscles (24). The effects of PGE2 on antral activity are absent in W/Wv mice which lack gastric ICC-IM, suggesting that these effects are mediated by receptors on ICC-IM (24). The chronotropic receptor expressed by ICC is the EP3 subtype (PTGER3) (24,25).

We investigated gastric electrical and mechanical activities of Lepob mice and observed that the abnormal patterns of activity were similar to behaviors caused by PGE2 administration. Therefore, we investigated the hypothesis that the gastric motor abnormalities seen in this diabetic animal model might be related to overproduction of PGE2, working through receptors in ICC that affect pacemaker activity. We used immunohistochemical, molecular and functional approaches to characterize enteric nerves, interstitial cell populations, and changes in the molecules that enhance PGE2 signaling and characterize electro-mechanical patterning of gastric muscles of Lepob.
mice. Our results show that \textit{Lep}^{ob} mice have slow waves of increased frequency and decreased amplitude. Areas of antrum were found with no resolvable pacemaker activity, and these electrical anomalies were associated with abnormal contractile patterns. We also found that increased expression of specific components of the prostaglandin pathway, including PTGES1 and PGE$_2$ receptors, which may underlie the observed changes in gastric motility patterns.
RESEARCH DESIGN AND METHODS

Animals

$Lep^{ob}$ diabetic mice and wild-type littermates (C57BL/6) were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). $Lep^{ob}$ mice (8-10 weeks old) had greater weights: 48±3g ($n=6$) versus 23±1g for wild-types ($n=5$; $P\leq0.0001$). Animals were anesthetized by isoflurane (Baxter, Deerfield, IL) and exsanguinated after cervical dislocation. Blood glucose was measured from tail tips using an Ascensia-Contour Blood Glucose Monitoring System (Bayer HealthCare LLC, Mishawaka, IN). $Lep^{ob}$ mice were hyperglycemic, 256±30mg/dl ($n=15$) versus 154±7mg/dl for wild-type mice ($n=10$; $P=0.014$). Stomachs from age-matched $Lep^{ob}$ mice and wild-type controls were removed and opened along the lesser curvature, and the antral mucosa removed by sharp dissection. All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The Institutional Animal Use and Care Committee at the University of Nevada, Reno approved procedures.

Electrophysiological Measurements

Antral muscles were pinned to the Sylgard elastomer floor of a recording chamber and perfused with 37°C KRB. Circular muscle cells were impaled with KCl filled glass microelectrodes (50-100MΩ). Transmembrane potentials were measured using an amplifier (Axon Instruments, Sunnyvale, CA) and digitized (Digidata 1300 series; Axon Instruments). Slow waves were recorded from 9 mapped regions of each antrum. Measurements of resting membrane potential (RMP), amplitude, frequency, half-maximal duration and inter-slow wave period (ISWP) were made using Clampfit 10.0 (Axon Instruments).
Post-junctional neural responses were recorded in response to electrical field stimulation (EFS; 0.3ms pulse duration, 1-20 Hz, train durations of 1s, 10-15 volts) delivered by a Grass S48 stimulator (Quincy, MA, USA).

**Isometric Force Measurements**

Standard isometric force measurements were performed as previously described (20). Details are provided in the supplementary materials.

**Video Imaging of Gastric Peristalsis**

Motility patterns of antrums were recorded as previously described (24). Specific details are provided in supplementary materials.

**Immunohistochemistry**

Immunohistochemistry of whole mount gastric preparations were performed as previously described (26). Specific details are provided in supplementary materials and supplemental table 1 for antibodies used.

**Western Analysis**

Western analysis was performed using standard techniques and as previously described (27). Details are provided in the supplementary materials.

**RNA Isolation and Real-Time Quantitative PCR**
RNA isolation and qPCR performed using standard techniques (28). Details provided in supplementary materials.

**Solutions and Drugs**

Details of solutions and drugs used are described in supplemental materials.

**Statistical Analysis**

Standard statistical analysis was performed using appropriate statistical tests. Details are described in supplementary materials.
RESULTS

Disruption of pacemaker activity in gastric antrums of Lep\textsuperscript{ob} diabetic animals

The resting membrane potential (RMP) for gastric antrums of wild-type mice averaged -66±4.0mV \((n=14)\), while the antrums of Lep\textsuperscript{ob} mice were significantly depolarized at -60±5.0mV \((n=16; P=0.001)\). Slow waves occurred in wild-type antrums with a frequency of 3.6±0.7min\(^{-1}\) (Fig. 1A and C; \(n=14\)), but the frequency of slow waves was increased in Lep\textsuperscript{ob} antral muscles to 5.2±1.9min\(^{-1}\) (Fig. 1B and C; \(n=16; P=0.0049\)). Slow waves from wild-type mice had an average amplitude of 29±6.0mV (Fig. 1A and D; \(n=14\)) but were only 19±8mV in Lep\textsuperscript{ob} antral muscles (Fig. 1B and D; \(n=16; P=0.0002\)). The inter-slow wave period (ISWP) was 9.8±3.7s in wild-type mice (Fig. 1A and E; \(n=14\)) and significantly reduced to 4±1.9s in Lep\textsuperscript{ob} mice (Fig. 1B and E; \(n=14; P<0.0001\)). The half-duration of slow waves in wild-type (4.4±1.5s; Fig. 1A and F; \(n=14\)) and Lep\textsuperscript{ob} antral muscles (3.2±2.3s; Fig. 1B and F) was not significantly different (\(n=16; P=0.1085\)). Of the 16 Lep\textsuperscript{ob} antrums studied, 2 had slow wave frequencies and 4 had amplitudes that were not different from the average values of slow waves in wild-type mice. All animals were included in the analysis of electrical parameters.

Intracellular recordings were made at nine specific regions, along the antral wall from greater to lesser curvature, to determine if the disruption in slow wave activity was widely distributed or a localized occurrence. Fig. 2A and B shows a diagrammatic representation of the preparation and the location of each recording site. Typical slow waves from each region are shown in Fig. 2C and D. In the gastric antrums of wild-type animals slow waves tended to have reduced amplitudes in the distal antrum compared to proximal regions (compare regions 7-9 with regions 1-3 in Fig. 2C). The slow wave pattern was more heterogenous in Lep\textsuperscript{ob} antrums, with some regions (e.g. 7 and 8) displaying an absence of slow waves (Fig. 2D). Summary data reveals significantly
increased slow wave frequencies and reduced amplitudes in numerous antral regions (Supplementary Fig. 3). Means±SD from the 9 recorded regions are shown in Supplemental Table 4.

**Post-junctional neural responses in gastric antrums of Lep\textsuperscript{ob} animals**

We recorded post-junctional neural responses to electrical field stimulation of intrinsic neurons in wild-type and Lep\textsuperscript{ob} antrums. No significant differences were observed in responses at 1 and 10Hz (see supplemental materials and Supplemental Fig. 2).

**Antral mechanical activity is decreased, and motility is disordered in Lep\textsuperscript{ob} diabetic mice**

Isometric force measurements revealed that phasic contractions of Lep\textsuperscript{ob} antrums were reduced in amplitude and increased in frequency in comparison to antral muscles of wild-type mice. Circular muscle contractions occurred at 3.0±1.1min\textsuperscript{-1} (Fig. 1G and I; \textit{n}=15) and generated 1.9±0.51mN of force in wild-type muscles (Fig. 1G and J; \textit{n}=8). Contractions of Lep\textsuperscript{ob} antrums occurred at 4.1±1.4min\textsuperscript{-1} (Fig. 1H and I; \textit{n}=14; \textit{P}=0.033) and generated significantly less force (0.85±0.37mN; Fig. 1H and J; \textit{n}=9; \textit{P} \leq 0.001).

Video imaging of antral muscles from wild-type or Lep\textsuperscript{ob} mice was also performed to determine changes in motility patterns (Supplementary Fig. 1A-C). Spatiotemporal maps were produced from video recordings (Supplementary Fig. 1D and E). Wild-type antral muscles demonstrated robust regular contractions at a frequency of 4min\textsuperscript{-1} (Supplementary Fig. 1D). This activity was weakened, irregular, and occurred at a higher frequency of 5.5min\textsuperscript{-1} in Lep\textsuperscript{ob} antrums (Supplementary Fig.1E).
**ICC Networks, NOS1+ Nerves and PDGFRα+ Cells in Wild-Type and Lepob Mice**

Images from the greater curvature are presented in Figure 3. Supplemental Figure 4 shows images from the lesser curvature. No differences were observed in ICC-MY between wild-type (B in both Fig. 3 and Supplemental Fig. 4; n=7) and Lepob mice (D in both Fig. 3 and Supplemental Fig. 4; n=10) and ICC-IM in wild-type (A in Fig. 3 and Supplemental Fig. 4; n=7) and Lepob antrums (C in Fig. 3 and Supplemental Fig. 4; n=10). Likewise, no differences were detected in the densities of NOS1+ nerve fibers in the circular muscle layer of wild-type (E in both Fig. 3 and Supplemental Fig. 4; n=3) and Lepob mice (G in both Fig. 3 and Supplemental Fig. 4; n=6). NOS1+ neurons were also unchanged in the myenteric plexus of antrums of wild-type (F in Fig. 3 and Supplemental Fig. 4; n=3) and Lepob mice (H in Fig. 3 and Supplemental Fig. 4; n=6).

PDGFRα+ cells had similar densities in antral circular muscles of wild-type (I in Fig. 3 and Supplemental Fig. 4; n=5) and Lepob mice (K in Fig. 3 and Supplemental Fig. 4; n=4). No differences in PDGFRα+ cells were detected in the myenteric region either (J in Fig. 3 and Supplemental Fig. 4; n=5; L in Fig. 3 Supplemental Fig. 4; n=4).

**Protein Expression in ICC, Enteric neurons and Smooth Muscle in Wild-Type and Lepob Mice**

Western analysis was performed to quantify possible changes in cell-specific proteins expressed in ICC (KIT), enteric neurons (NOS1 and UCHL1), smooth muscle (MYH11), and also in pre- and post-synaptic proteins (SNAP25, syntaxin and DLG4). No significant differences were observed in any of these proteins between wild-type (n=4) and Lepob antrums (n=3; Fig. 4).
Effects of the PTGS2 Inhibitor Valdecoxib on Electrical and Mechanical Activities of *Lep*<sup>ob</sup> Gastric Antrums

Slow waves from *Lep*<sup>ob</sup> antrums are similar in waveform to wild-type slow waves after PGE<sub>2</sub> application (23,24). Thus, we recorded slow waves from *Lep*<sup>ob</sup> antrums before and in the presence of the PTGS2 inhibitor, valdecoxib (Fig. 5A, B and C). Under control conditions, *Lep*<sup>ob</sup> antrums exhibited slow waves with a frequency of 6.7±0.8min<sup>-1</sup> and an amplitude of 23±7.0mV (Fig. 5B, D and E; n=6). (NB: Recordings were made from region 5). Valdecoxib (1µM), reduced slow wave frequency to 5.2±1.1min<sup>-1</sup> (Fig. 5C and D; P=0.0059) and slow wave amplitude increased to 27±4.0mV (Fig. 5C and E; P=0.0161). Half-maximal duration of slow waves, ISWP and RMP were not significantly affected by valdecoxib. Like slow waves, the amplitudes of phasic contractions increased, and contraction frequency decreased after valdecoxib application. Contractile force generated by *Lep*<sup>ob</sup> antrums averaged 1.04±0.51mN and occurred at 3.8±1.5min<sup>-1</sup> (Supplemental Fig. 5A-D). In valdecoxib (1µM), contractile force increased to 2.15±1.25mN (Supplemental Fig. 5B and D; P<0.001, n=4) and frequency decreased to 2.5±0.8min<sup>-1</sup> (Supplemental Fig. 5B and C; P=0.001, n=5).

Valdecoxib also affected wild-type muscles. Slow waves were reduced in frequency from 3.3±0.8min<sup>-1</sup> to 2.7±0.8min<sup>-1</sup> (Fig. 5F, G and H; P=0.003; n=6). The ISWP increased from 11.9±5.5s to 18.5±7.4s (P=0.016; n=6). However, no differences were detected in amplitude (Fig. 5F, G and I), half-maximal duration or RMP. Valdecoxib (1µM) also influenced contractile activity in wild-type animals, reducing the frequency from 3.2±0.5min<sup>-1</sup> to 2.7±0.4min<sup>-1</sup> (Supplemental Fig. 5E, F and G; P=0.001; n=7). A small increase in contractile amplitude in *Lep*<sup>ob</sup> antrums did not reach statistical significance (Supplemental Fig. 5E, F and H; n=6).
Video imaging revealed that PTGS2 inhibition also affected motility in \textit{Lep}^{ob} antrums. The spatiotemporal map in Supplemental Fig. 5I shows that \textit{Lep}^{ob} antrums exhibit frequent, weak contractions. After application of valdecoxib (1\mu M; 20 min), contractions became more robust and occurred at a frequency of 2.8 min^{-1} (Supplemental Fig. 5J).

**Changes in mRNA Expression of Key Genes Associated with Prostaglandin Signaling in Gastric Antrums From \textit{Lep}^{ob} Mice**

Chronic, sub-clinical inflammation is associated with type 2 diabetes and chronic inflammation has been shown to underlie the development of insulin resistance in animal models of type 2 diabetes (29). As inflammation causes PTGS2 upregulation (30), qPCR was performed to determine whether PTGS2 and other enzymes involved in prostaglandin synthesis, its breakdown, and receptors involved in mediating its biological response were changed in \textit{Lep}^{ob} antrums. \textit{Ptgs2} was significantly upregulated in \textit{Lep}^{ob} antrums (Fig. 6; \( n=4; \ P=0.0003 \)). Likewise, expression of the terminal enzyme for PGE$_2$ synthesis, \textit{Ptges}, was also significantly increased (Fig. 6; \( n=4; \ P=0.0004 \)).

Expression of \textit{Ptger1}, \textit{Ptger2} and \textit{Ptger3} (prostaglandin E2 receptor subtypes EP1, EP2 and EP3), were also increased in \textit{Lep}^{ob} antrums (Fig. 6; \( n=4; \ P=0.0007, 0.0005 \) and 0.0007, respectively), while expression of \textit{Ptger4} (prostaglandin E2 receptor subtype EP4) was not altered. We also assessed the mRNA expression of \textit{Ptgfr} and \textit{Ptgir}, receptors for PGF$_{2\alpha}$ and PGI$_2$, and both were significantly increased in \textit{Lep}^{ob} antrums (Fig. 6; \( n=4; \ P=0.0019 \) and 0.0002, respectively).

Finally, \textit{Hpgd} (15-hydroxyprostaglandin dehydrogenase), an enzyme involved in the breakdown of prostaglandins, was increased in diabetic antrums (Fig. 6; \( n=4; \ P=0.0018 \)). Other enzymes involved in production and breakdown of prostaglandins, \textit{Ptgs1} (prostaglandin-endoperoxide synthase 1) and \textit{Ptgr1} (Prostaglandin Reductase 1) respectively, were not changed in \textit{Lep}^{ob}.
antrums. Since valdecoxib, a specific PTGS2 inhibitor, had significant effects on antral electrical and contractile behavior of Lep<sup>ob</sup> antrums (Fig. 5 and Supplemental Fig. 5), upregulation of gene transcripts likely plays a significant role in the changes in gastric motor activity in these diabetic animals.
DISCUSSION

Patients with diabetes often suffer significant GI symptoms, including gastroparesis, which can significantly impair their quality of life (31). Previous studies suggested that an enteric neuropathy is the underlying cause of gastroparesis. A decrease in NOS1 containing inhibitory neurons, and changes in cholinergic excitatory neurons, have been reported in the GI tracts of diabetic animal models (2,32). Reduced numbers of ICC have also been observed in diabetic GI muscles (5,6,33). More recently, increased numbers of ICC have been reported in Lepr\textsuperscript{db} mice with rapid gastric emptying (17). Thus, reports on the underlying changes in enteric nerves and ICC vary considerably. Despite the number of studies that have reported changes in neuronal or ICC populations, little information has been garnered on functional changes that underlie gastroparesis associated with type 2 diabetes.

In this study, we utilized Lepr\textsuperscript{db} mice, a type 2 diabetic animal model, to examine functional changes that occur in gastric electrical and mechanical activities. Electrical slow waves were increased in frequency and reduced in amplitude and associated contractions were more frequent and weaker in diabetic antrums compared to wild-type animals. Furthermore, electrical recordings made from 9 defined antral recording sites revealed that slow waves were significantly reduced and, in some animals, could not be resolved at several sites in Lepr\textsuperscript{db} antral muscles. These data would suggest that slow wave generation in some sites becomes ineffective and propagation of slow waves, necessary for coordinated gastric peristalsis and efficient chemo-mechanical breakdown of gastric contents, does not occur normally. Such loss of function could lead to delayed gastric emptying. This information was only detected following systematic electrical recordings from an array of multiple sites and would be undetected if single recordings sites were utilized or larger tissue preparations were employed (i.e. isometric force measurements).
We found no evidence that the densities of NOS1+ neurons and ICC were altered in \textit{Lep}^{ob}\textit{mice}. Recently it has been reported that gene transcripts for NOS1 and VACHT, bio-markers for inhibitory and excitatory neurons, were slightly increased in gastric tissues from non-diabetic idiopathic gastroparesis patients (34). This study also revealed no significant changes in gene transcripts specific for ICC (e.g. \textit{Kit} and \textit{Ano1}) in these patients, although decreased \textit{Pdgfra}, a marker for PDGFR\alpha+ cells, was observed. In \textit{Lep}^{ob} mice, the density of PDGFR\alpha+ cells, another specialized interstitial cell in GI muscles (35), appeared normal, as previously reported for gastric muscles of human patients with diabetes (36). Thus, considerable discrepancy exists in reports describing changes in enteric nerve and interstitial cell populations depending on the animal model or type of diabetes studied.

Rather than gross morphological or structural changes in neurons or interstitial cells, changes in slow wave activity in \textit{Lep}^{ob} mice appear to be due to abnormal prostaglandin production and responses. The conclusion that prostaglandins are involved, stems from the observation that PTGS2 inhibition by valdecoxib resulted in slow wave frequency and amplitude returning towards normal values and contractile activity becoming more robust, similar to wild-type controls. Valdecoxib, at the concentration used in the present study, is reported to be a potent and selective inhibitor of PTGS2 versus PTGS1 \textit{in vitro} and \textit{in vivo} (37). Thus, the normalization of slow wave activity in \textit{Lep}^{ob} mice is likely be due to inhibition of PTGS2 rather than PTGS1. Application of PGE$_2$ to wild-type antrums results in increased slow wave frequency and reduction in amplitude (23,24), mimicking \textit{Lep}^{ob} antrum activity. The positive chronotropic effects of prostaglandins may occur via PTGER3 as specific agonists of this receptor increased pacemaker activity in isolated ICC and tissues (25). Stretch-dependent experiments on the gastric antrum revealed that length ramps produced membrane depolarization and chronotropic effects on pacemaker activity (38).
These stretch-dependent responses were inhibited by indomethacin and absent in PTGS2-deficient mice. Stretch-dependent responses on W/W\textsuperscript{v} mice, that lack gastric ICC-IM, were absent. These data suggested that antral ICC-IM are mechanosensitive and chronotropic responses to length ramps were mediated via prostaglandin production by these cells (38).

Diabetes is associated with chronic, sub-clinical inflammation and is considered an inflammatory disease (29). Macrophage-derived inflammation in adipose tissue can trigger insulin resistance (39). Changes in macrophage populations are reported to occur in diabetic and idiopathic gastroparesis. In the stomachs of these patients there was loss of CD206\textsuperscript{+} anti-inflammatory macrophages and associated loss of ICC, suggesting that CD206\textsuperscript{+} macrophages are cryoprotective (40,41). However, we did not observe a loss of antral ICC in the present study and cannot say whether an inflammatory response exists in Lep\textsuperscript{ob} antrums. To explain the changes in gastric motor activity of Lep\textsuperscript{ob} stomachs, we examined the expression of genes involved in the synthesis and breakdown PGE\textsubscript{2}, and its receptors, as these components of the prostaglandin pathway affect gastric motor activity (20,23,24). We found that Ptgs2 gene transcripts were up-regulated in Lep\textsuperscript{ob} antrums. Furthermore, Ptges, which encodes microsomal prostaglandin E\textsubscript{2} synthase, is also highly augmented in Lep\textsuperscript{ob} antrums, providing an additional mechanism that could contribute to the apparent increase in PGE\textsubscript{2} activity. PTGES is a terminal prostaglandin E synthase that converts PGH\textsubscript{2} to PGE\textsubscript{2} (see Fig. 7). Unlike other prostaglandin E syntheses (PTGES2 and PTGES3), which are constitutively expressed, PTGES is induced by proinflammatory stimuli, and has been observed to be upregulated in various inflammatory diseases, such as inflammatory bowel disease (42). In addition to PGE\textsubscript{2} synthesis enzymes, we found that gene transcripts for multiple PGE\textsubscript{2} receptors (Ptger1, Ptger2, Ptger3) were also increased in diabetic antrums. This indicates that, along with increased PGE\textsubscript{2} production, the tissue also has a greater capacity to respond to
PGE$_2$. Notably, *Ptger3*, encoding PTGER3, is one of the upregulated gene transcripts and, as previously mentioned, it has been implicated as the receptor involved in the gastric motor effects of PGE$_2$ (24,25). Additionally, *Ptgfr* and *Ptgir*, which encode the receptors for PGF$_{2\alpha}$ and PGI$_2$ respectively, were increased. Both PGF$_{2\alpha}$ and PGI$_2$ are known to affect gastric motor activity but PGF$_{2\alpha}$ does not affect gastric rhythmicity (43) and PGI$_2$ had a much weaker effect on motility than PGE$_2$ (44).

In this study, we did not observe changes in NOS1$^+$ enteric neurons or ICC, contrasting with some previously published findings (2,6,45). An explanation for this disparity is the different approaches used to identify and quantify particular gastrointestinal cell types. In previous studies, cross section preparations of muscle tissues have simply been used to quantify cells of interest. This technique has received criticism and is currently generally not a well-accepted approach to determine changes in gastrointestinal muscles (46). Our immunohistochemical analysis was performed using confocal microscopy on six randomly selected areas of whole mounts imaging the entire muscle thickness. Thus, the approach taken in this study should accurately reflect the genuine situation of cellular networks of *Lep$^{ob}$* antrums. Another possible reason is that we used an animal model of type 2 diabetes, while the majority of previously studies have investigated type 1, or streptozotocin-induced diabetes.

While we have observed profound changes in the prostaglandin pathway in *Lep$^{ob}$* antrums, we also found that PTGS2 inhibition affected slow wave activity in wild-type mice. It has previously been shown that PTGS2 is constitutively expressed in antral tissues of wild-type mice (20). Since PTGS2 inhibition altered pacemaker activity and contractile responses in *Lep$^{ob}$* and wild-type antrums, this suggests ongoing PGE$_2$ production in wild-type tissues, although production is upregulated in *Lep$^{ob}$* antrums.
In conclusion, pacemaker activity in the gastric antrum is disrupted in a type 2 diabetic mouse model. Slow waves were increased in frequency and reduced in amplitude, resulting in phasic contractions that were augmented in frequency and greatly reduced in amplitude. These changes may be involved in the pathogenesis of diabetic gastroparesis. The disruption in pacemaker activity is likely the result of increased prostaglandin synthesis enzymes and receptors for PGE$_2$. These findings raise the exciting possibility of a novel treatment for diabetic gastroparesis by inhibition of prostaglandin synthesis.
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Figure 1 - Frequency of pacemaker activity was greater, and amplitude reduced, in $Lep^{ob}$ antrums. 

$A$ and $B$: Representative intracellular microelectrode recordings from the antrums of wild-type ($A$) and $Lep^{ob}$ ($B$) mice. Note the depolarized membrane potential, increased frequency and decreased amplitude of slow waves in $Lep^{ob}$ antrums. Timescale in $B$ applies to both $A$ and $B$. $C$-$F$: Summarized data demonstrating differences in slow wave parameters between wild-type (white bars) and $Lep^{ob}$ antrums (black bars). $C$: increase in slow wave frequency, $D$: reduction in slow wave amplitude, $E$: reduction in inter-slow wave period and $F$: reduction in half maximal duration of slow waves (unpaired Student's t-test). $G$ and $H$: Representative isometric force recordings from the antrums of wild-type ($G$) and $Lep^{ob}$ ($H$) mice. $I$-$J$: Summarized data showing increased frequency ($I$) and reduced amplitude ($J$) of contractions in $Lep^{ob}$, as compared to wild-type (unpaired Student's t-test).
**Figure 2** - Mapping of pacemaker activity throughout the antrums of wild-type and *Lep<sup>ob</sup>* animals.

*A and B*: Diagrammatic illustrations showing the gastric antrum muscle preparation and the regions from which electrical recordings were obtained. *A*: Antrums were separated from intact stomachs and opened along the lesser curvature (LC). *B*: Isolated antrums were pinned flat, the mucosa removed, and intracellular microelectrode recordings made from proximal antrum (regions 1-3), mid antrum (regions 4-6) and the terminal antrum (regions 7-9). *C* and *D*: Typical electrical activity recorded from the circular muscle layers of each of the nine regions of wild-type (*C*) and *Lep<sup>ob</sup>* antrums (*D*). Note that membrane potentials were more depolarized and pacemaker activity was higher in frequency and decreased in amplitude in *Lep<sup>ob</sup>* antrums compared to wild-type controls. In two regions of the *Lep<sup>ob</sup>* antrum no slow wave activity was recorded.
**Figure 3** - Networks of ICC, NOS1+ nerves and PDGFRA+ cells along the greater curvature of antrums from wild-type and *Lep*<sup>ob</sup> animals. Representative images of ICC (A-D), NOS1+ neurons (E-H) and PDGFRA+ cells (I-L). A, E and I show ICC, NOS1+ nerve fibers and PDGFRA+ cells, respectively, within the circular muscle layer of a wild-type antrum. C, G and K show ICC, NOS1+ nerve fibers and PDGFRA+ cells, respectively, within the circular muscle layer of a *Lep*<sup>ob</sup> antrum. B, F and J show ICC, NOS1+ neurons and PDGFRA+ cells, respectively, in the myenteric plexus region of a wild-type antrum. D, H and L show ICC, NOS1+ neurons and PDGFRA+ cells, respectively, in the myenteric plexus region of a *Lep*<sup>ob</sup> antrum. Scale bar in L applies to all panels.
Figure 4 - Western analysis for proteins relating to ICC, enteric nerves and smooth muscle cells in antrums from *Lep*<sup>ob</sup> and wild-type animals. *A*: Representative Western blots comparing the expression of various proteins associated with ICC (KIT; DLG4), enteric nerves (NOS1; UCHL1, SNAP 25, and syntaxin) and smooth muscle (MYH11) in wild-type and *Lep*<sup>ob</sup> mice, respectively. Each band is demarcated by a boundary box to illustrate that they were from different gels or different parts of the same gel. Bands are the most representative from the gastric antrums of three different animals. GAPDH was used as a control housekeeping protein to which the other proteins were compared. Three separate GAPDH blots, one from each animal, are shown. *B*: Western analysis bands from wild-type and *Lep*<sup>ob</sup> antrums were quantified by densitometry and expressed relative to GAPDH. The summary graph demonstrates that the major proteins associated with ICC, enteric neurons and smooth-muscle were unchanged in the antrums of *Lep*<sup>ob</sup> compared to wild-type animals (unpaired Student's *t*-test).
Figure 5 - PTGS2 inhibition normalizes disrupted antral pacemaker activity in Lep^{ob} antrums. A: Continuous intracellular microelectrode recording from a Lep^{ob} antrum under control conditions (no drugs) and after the addition of the PTGS2 inhibitor valdecoxib (1 µM). Slow waves increased in amplitude and decreased in frequency. B and C: Intracellular recordings at a faster sweep speed before (B) and in the presence of valdecoxib (C). Traces show the changes in slow wave parameters. D and E: Summary of slow wave changes in slow wave frequency (D) and slow wave amplitude (E). F and G: Typical recordings of wild-type antrum under control conditions (F) and after the addition of valdecoxib (G). H and I: Summary of the effects of valdecoxib on slow wave frequency (H) and (I) amplitude. Valdecoxib decreased frequency but had no effect on amplitude (paired Student's t-test).
**Figure 6** – qPCR comparing the expression of several key genes involved in prostaglandin synthesis and signaling in antrums from wild-type and *Lep*<sup>ob</sup> mice. Bar graph depicts the fold change in prostaglandin-related gene transcripts. Several gene products were shown to be significantly upregulated in *Lep*<sup>ob</sup> antrums compared to wild-type controls: terminal PGE<sub>2</sub> synthase (*Ptges*), Prostaglandin E2 receptor subtypes EP1, EP2 and EP3 (*Ptger1, Ptger2, Ptger3*), Prostaglandin-Endoperoxide Synthase 2 (*Ptgs2*), Prostaglandin F receptor (*Ptgfr*), Prostaglandin I receptor (*Ptgir*) and 15-Hydroxyprostaglandin Dehydrogenase (*Hpgd*). Prostaglandin E2 receptor subtypes EP4 (*Ptger4*), Prostaglandin Reductase 1 (*Ptgr1*) and Prostaglandin-Endoperoxide Synthase 1 (*Ptgs1*) did not differ significantly in gene transcript expression between *Lep*<sup>ob</sup> antrums and wild-type controls (unpaired Student's *t*-test).
Figure 7 – Diagram depicting the prostanoid synthesis pathway and the effects of PGE$_2$ on its receptor subtypes. Highlighted boxes identify prostaglandin-related genes that are upregulated in the gastric antrums of $Lep^{ob}$ tissues. We hypothesize that upregulation of PTGES, encoding microsomal prostaglandin E$_2$ synthase, results in increased levels of PGE$_2$, which in turn results in abnormal antral slow wave and contractile activity. The PTGER1-3 receptors are also upregulated and likely play a role in the altered gastric motor activity.
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Figure 1 - Frequency of pacemaker activity was greater, and amplitude reduced, in Lepob antrums. A and B: Representative intracellular microelectrode recordings from the antrums of wild-type (A) and Lepob (B) mice. Note the depolarized membrane potential, increased frequency and decreased amplitude of slow waves in Lepob antrums. Timescale in B applies to both A and B. C-F: Summarized data demonstrating differences in slow wave parameters between wild-type (white bars) and Lepob antrums (black bars). C: increase in slow wave frequency, D: reduction in slow wave amplitude, E: reduction in inter-slow wave period and F: reduction in half maximal duration of slow waves (unpaired Student's t-test). G and H: Representative isometric force recordings from the antrums of wild-type (G) and Lepob (H) mice. I-J: Summarized data showing increased frequency (I) and reduced amplitude (J) of contractions in Lepob, as compared to wild-type (unpaired Student's t-test).
Diabetes

C. Wild-type

No. 1
No. 2
No. 3
No. 4
No. 5
No. 6
No. 7
No. 8
No. 9

10 sec

D. Lep<sup>ob</sup>

No. 1
No. 2
No. 3
No. 4
No. 5
No. 6
No. 7
No. 8
No. 9

10 sec
Figure 3 - Networks of ICC, NOS1+ nerves and PDGFRA+ cells along the greater curvature of antrums from wild-type and Lepob animals. Representative images of ICC (A-D), NOS1+ neurons (E-H) and PDGFRA+ cells (I-L). A, E and I show ICC, NOS1+ nerve fibers and PDGFRA+ cells, respectively, within the circular muscle layer of a wild-type antrum. C, G and K show ICC, NOS1+ nerve fibers and PDGFRA+ cells, respectively, within the circular muscle layer of a Lepob antrum. B, F and J show ICC, NOS1+ neurons and PDGFRA+ cells, respectively, in the myenteric plexus region of a wild-type antrum. D, H and L show ICC, NOS1+ neurons and PDGFRA+ cells, respectively, in the myenteric plexus region of a Lepob antrum. Scale bar in L applies to all panels.
Valdecoxia (1 μM)
SUPPLEMENTAL MATERIAL

Isometric Force Measurements

Standard organ baths techniques were used to measure contractile activity in strips of wild-type and Lep
ob antrum. Muscle strips were cut parallel to the circular layer and attached, via fine silk suture thread, to an isometric force transducer (Fort10g, World Precision Instruments, Sarasota, FL) at one end and to a fixed support at the other. The strips were placed in organ baths that were continually perfused with oxygenated KRB maintained at 37°C. Passive tension of 2-3mN was applied to the antral muscle strips. The muscle strips were allowed to equilibrate in the organ baths for 1hr during which spontaneous, rhythmic contractions developed.

Video Imaging of Gastric Peristalsis

Antral muscle sheets were pinned flat to the base of a Sylgard elastomer (Dow Corning Corp, Midland, MI) coated dish, circular muscle layer facing upwards. Gastric muscles were perfused with oxygenated KRB at 37°C. The recording and analysis of videos has previously been described (1). In brief, antral movements were monitored by video recordings (4min; Fig. 1C), using a high-definition video camera (DMK 31AF03, ImagingSource, Charlotte, NC) and AstroIIDC software (ASC, Calgary, Alberta, Canada). Peristaltic contractions resulted in indentations of the muscle sheet edges, which could be detected and used to produce spatiotemporal maps using Volumetry software (Grant Hennig; Supplementary Fig. 1D and E). The process by which Volumetry generates spatiotemporal maps from video recordings has been detailed previously (1). Edge tracking techniques and black markers placed on the surface of the muscles were used to track muscle contractions. The frequency and amplitude (i.e. contractile displacement) of antral contractions were calculated from the spatiotemporal maps.
**Immunohistochemistry**

Whole mounts of gastric antrum were stretched to 110% of resting length and width before being fixed in paraformaldehyde (4% w/v; 10min; 4°C). Tissues were washed overnight in phosphate buffered saline (PBS; 0.01M, pH7.2) and incubated in bovine serum albumin (BSA; 1%, 1.5hr, room temperature) to reduce non-specific antibody binding. Primary antibody incubations were for 48h, 4°C (see Supplemental Table 1 for antibody details), diluted in 0.5% Triton-X 100. Tissues were washed with PBS (4x1hr). Immunoreactivity was detected using the appropriate Alexa Fluor 488 antibodies (1:1,000 in PBS; 1hr, room temperature). Control tissues were prepared by omitting either primary or secondary antibodies from the incubation solution. After washing with PBS (overnight, 4°C), whole mounts were mounted on glass slides and examined with an LSM 510 Meta confocal microscope (Zeiss, Oberkochen, Germany). Images were then deconvolved (AutoQuantX, MediaCybernetics, MD). Confocal micrographs are digital composites of image stacks that were constructed using Zeiss LSM 5 Image Examiner software and converted to TIFF files.

**Western Analysis**

Total protein was extracted from \( Lep^{ob} \) and wild-type antral muscles. Protein concentration of isolated muscle extracts was determined by the Bradford assay (Bio-Rad, Richmond, CA). A total of 30µg of protein from each lysate was used for the blot. Proteins were subjected to 10% SDS-PAGE, transferred to a nitrocellulose membrane and probed with the primary antibodies listed in supplemental Table 2. After washing, the blot membrane was incubated with alkaline phosphatase-conjugated immunoglobulin G secondary antibody (Santa Cruz Biotechnology, Inc.,
Dallas, Texas). After another wash, the color development by NBT/BCIP (Roche Diagnostics, Mannheim, Germany) was stopped. The amount of proteins on the blot was analyzed using Quantity One 4.5.1 software (Bio-Rad, Hercules, California). ImageJ software (NIH, Bethesda, MD) was used to calculate the relative densities of protein compared with housekeeper glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

**RNA Isolation and Real-Time Quantitative PCR**

Total RNA was isolated from the tunica muscularis of Lep\textsuperscript{ob} and wild-type antrums (following removal of the mucosa and submucosa) using TRIzol Reagent (Invitrogen, Carlsbad, CA) as per the manufacturer’s protocol; tissues were homogenized using a tissue grinder and 21-gauge sterile syringe during the lysis phase of the protocol. Final product was eluted with 25µl diethylpyrocarbonate (DEPC)-treated water. Concentration and purity of RNA was measured using a ND-1000 Nanodrop Spectrophotometer (Nanodrop, Wilmington, DE). First strand cDNA was synthesized from 100ng of each RNA using qScript™ cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD) in a 5X reaction buffer containing optimized concentrations of MgCl\textsubscript{2}, dNTPs (dATP, dCTP, dGTP, dTTP), recombinant RNase inhibitor protein, qScript reverse transcriptase, random primers, oligo(dT) primer and stabilizers. This was followed by heat inactivation. Primers were designed to investigate the expression of various components of the prostaglandin synthesis pathway and prostaglandin receptors (see supplemental Table 3 for primer details). Quantitative PCR (qPCR) was performed with the same primers used for PCR using SYBR green chemistry on the 7900 HT Real Time PCR System (Applied Biosystems, Carlsbad, CA). cDNA was prepared from four wild-type and four Lep\textsuperscript{ob} antrums and each cDNA was tested in triplicate replicates. Negative (no template) controls were included in each qPCR
run. Melting curve analysis was performed using the manufacturer’s standard protocol with no evidence for primer dimer or nonspecific products. Regression analysis using the serial 10-fold dilutions cDNA was used to generate standard curves. Unknown amounts of messenger RNA (mRNA) were plotted relative to the standard curves for each set of primers and graphically plotted using Microsoft Excel. A regression line with a correlation coefficient ($R^2$ value) of >0.98 and primer efficiencies of 95-105% were only accepted for analysis. This gave transcriptional quantification of each gene relative to GAPDH standard after log transformation of the corresponding raw data. Differences between normalized wild-type and Lep$^{ob}$ samples were assessed using a Students t-test assuming unequal variance ($P>0.05$).

**Solutions and Drugs**

Recording chambers for physiological experiments were perfused with KRB containing (in mmol/L): NaCl, 120.35; KCl, 5.9; NaHCO$_3$, 15.5; NaH$_2$PO$_4$, 1.2; MgCl$_2$, 1.2; CaCl$_2$, 2.5; and glucose, 11.5. The pH of the KRB was maintained at 7.3-7.4 when bubbled with 97% O$_2$-3% CO$_2$ at 37±0.5°C. After pinning, antral tissues were left to equilibrate for 1hr before experiments were started. Valdecoxib (Tocris Bioscience, Bristol, UK) was used to inhibit PTGS2 and was dissolved in DMSO at a stock concentration of 10mM before being added to KRB and perfused at a final concentration of 1µM.

**Statistical Analysis**

Significance was calculated by appropriate statistical tests. Data are expressed as means±SD. The use of “n” refers to the number of animals utilized. $P$ values <0.05 were considered statistically significant. In figures, *, **, ***, **** represent $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, $P \leq$
0.0001, respectively. GraphPad Prism 7 was used for analysis and graph production (GraphPad Software, Inc., La Jolla, CA). All figures were arranged using Corel Draw X4 (Corel Corp., Mountain View, CA).

SUPPLEMENTAL DATA

Post-junctional neural responses in gastric antrums of \textit{Lep}^{ob} animals
Enteric inhibitory neural responses in antrums of mice consist predominantly of a fast-purinergic inhibitory junction potential (IJP) (25). Single pulses (0.3ms duration) caused IJP amplitudes averaging 12±2.0mV with a half-maximal duration of 0.7±0.2s in wild-type mice (Supplementary Fig. 2A, C and D; n=3) and 11±2.0mV with a half-maximal duration of 0.57±0.1s in Lep\textsuperscript{ob} antral muscles (Supplementary Fig. 2B, C and D; n=5). Stimulating at 10Hz (0.3ms;1s) caused IJPs averaging 11±1.0mV with half-durations of 1.7±0.4s in wild-type mice (Supplementary Fig. 2A, C and D; n=4) and 10±4.0mV with half-durations of 1.5±0.1s in Lep\textsuperscript{ob} mice (Fig. 2B, C and D; n=5). Thus, significant differences in post junctional neural responses were not detected in Lep\textsuperscript{ob} antrums. However, since antrums of Lep\textsuperscript{ob} mice were depolarized by 6 mV compared to controls, the amplitude of IJPs in Lep\textsuperscript{ob} tissues could be considered to be reduced.

SUPPLEMENTAL FIGURE LEGENDS
**Supplemental Figure 1** - Disrupted antral propagating motor patterns in \( Lep^{ob} \) gastric antrums.

*A:* Image of intact stomach with gastric anatomical regions indicated (LC and GC stand for lesser curvature and greater curvature, respectively). The method of dissection of the stomach is marked by the dashed line and scissors. The stomach was cut open along the lesser curvature and the fundus removed. *B:* The muscle sheet was then pinned flat and the mucosa removed by sharp dissection. Black markers were placed on the surface of the circular muscle to allow monitoring of gastric wall contractions. The vertical dashed line indicates the axis of the greater curvature, while the horizontal dashed line and scissors indicates where corpus and antrum were separated. *C:* Image of the preparation after corpus and antrum were dissected apart (Scale bar in *A* applies to panels *A*-C). *D* and *E:* Video recordings, 4 minutes in length, were made in order to observe the contractile activity of the muscle sheet. Spatiotemporal maps were generated from antral regions by tracking indentation of muscle edges, during circular muscle contractions. Each contraction produces a vertical black band on the spatiotemporal map. Left to right on each spatiotemporal map represents time, while top to bottom represents space (proximal antrum to distal antrum). *D:* A spatiotemporal map generated from the antral region of a wild-type mouse showing robust contractions at a frequency of 4 min\(^{-1}\). *E:* A spatiotemporal map from the antrum of a \( Lep^{ob} \) mouse; contractions were much weaker than wild-type control, producing small movements and therefore only faint vertical black bands were observed (apart from one intense contraction towards the end of the 4-minute recording). The weaker contractions of the \( Lep^{ob} \) antrum occurred at 6 min\(^{-1}\).
Supplemental Figure 2 – Post-junctional neural responses were unaffected in $Lep^{ob}$ antrums compared to wild-type controls. A: Typical post-junctional neural responses to electrical field stimulation (EFS; 0.3ms duration pulses delivered at 1Hz (arrow) and 10Hz (horizontal bar) for 1 second) consisted of membrane hyperpolarization termed an inhibitory junction potential (IJP). B: In $Lep^{ob}$ antrums, EFS evoked IJPs that were similar in amplitude and half-maximal duration as controls. C and D: Summarized data showing no statistical difference in inhibitory neural responses between wild-type control (white bars) and $Lep^{ob}$ antrums (black bars) (unpaired Student's $t$-test).
Supplemental Figure 3 – Summarized slow wave parameters from 9 intracellular recording sites of antrums from wild-type controls and \textit{Lep}^{ob} animals. \textit{A}: Slow wave frequency showing statistically significant differences in most regions of \textit{Lep}^{ob} (black bars) compared to wild-type animals (white bars). \textit{B}: Slow waves amplitudes were also significantly smaller in most regions of the gastric antrums of \textit{Lep}^{ob} animals compared to wild-type controls (unpaired Student's t-test).
**Supplemental Figure 4** - Networks of ICC, NOS1\(^+\) nerves and PDGFRA\(^+\) cells along the lesser curvature of antrums from \textit{Lep}\(^{ob}\) and wild-type animals. \textit{A-D}: Representative images of ICC, \textit{E-H}: NOS1\(^+\) neurons and \textit{I-L}: PDGFRA\(^+\) cells. \textit{A, E} and \textit{I} show ICC, NOS1\(^+\) nerve fibers and PDGFRA\(^+\) cells, respectively, within the circular muscle layer of a wild-type antrum; \textit{C, G} and \textit{K} show ICC, NOS1\(^+\) nerve fibers and PDGFRA\(^+\) cells, respectively, within the circular muscle layer of a \textit{Lep}\(^{ob}\) antrum. \textit{B, F} and \textit{J} show ICC, NOS1\(^+\) neurons and PDGFRA\(^+\) cells, respectively, in the myenteric plexus region of a wild-type antrum. \textit{D, H} and \textit{L} show ICC, NOS1\(^+\) neurons and PDGFRA\(^+\) cells, respectively, in the myenteric plexus region of a \textit{Lep}\(^{ob}\) antrum. Scale bar in \textit{L} applies to all panels.
**Supplemental Figure 5** - PTGS2 inhibition normalizes phasic contraction force and frequency and restores motility patterns of Lep^ob antrums. *A* and *B*: Phasic contractile activity of the circular layer of a Lep^ob antrum before (*A*) and after (*B*) valdecoxib (1 µM). *C* and *D*: Summary of the changes in frequency (C) and isometric force (D) of phasic antral contractions of Lep^ob muscles before and after valdecoxib. *E-H*: In comparison, phasic contractions of the circular layer of a wild-type antrum before (*E*) and after (*F*) valdecoxib. *G* and *H*: Summarized data of the changes in frequency (G) and contractile force (H) of wild-type controls before and after valdecoxib. Note that changes of Lep^ob antrums were significantly greater than wild-type controls. Statistically significant changes are indicated (paired Student’s *t*-test). *I* and *J*: Spatiotemporal maps show normalization of gastric antrum motility patterns of a Lep^ob antrum after valdecoxib (1 µM). Spatiotemporal map of Lep^ob antrum prior to (*I*) and after (*J*) addition of valdecoxib (1µM). Note this map was from the same recording shown in figure 1.
SUPPLEMENTAL REFERENCES

1. Forrest AS, Hennig GW, Jokela-Willis S, Park CD, Sanders KM. Prostaglandin regulation of gastric slow waves and peristalsis. Am J Physiol Gastrointest Liver Physiol. 2009;296(6):G1180-90.
## SUPPLEMENTAL TABLES

### Supplemental Table 1 – Details of Primary Antibodies Used for Immunohistochemistry

| Antibodies   | Resource                                      | Mono- or poly-clonal antibodies | Host   | Dilution |
|--------------|-----------------------------------------------|----------------------------------|--------|----------|
| Anti-mSCF-R  | R&D Systems Inc., Minneapolis, MN, USA        | Poly                             | goat   | 1:500    |
| Anti-NOS1    | Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA | Poly                             | Rabbit | 1:500    |
| Anti-mPDGFRα | R&D Systems Inc., Minneapolis, MN, USA        | Poly                             | goat   | 1:100    |

Note: mSCF-R: mouse stem cell factor receptor; mPDGFRα: mouse platelet derived growth factor receptor α; NOS1: nitric oxide synthase 1.
**Supplemental Table 2** – Details of Antibodies Used for Western Blots

| Antibodies | Resource |
|------------|----------|
| GAPDH      | Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA |
| mSCF-R     | R&D Systems Inc., Minneapolis, MN, USA |
| UCHL1      | UltraClone Limited, Isle of Wight, England, UK |
| NOS1       | Gift from Dr. Piers Emson, Molecular Science Group, Cambridge, UK |
| SNAP25     | Covance, Princeton, NJ, USA |
| Syntaxin   | Sigma-Aldrich, Saint Louis, MO, USA |
| DLG4       | Millipore, Billerica, MA, USA |
| MYH11      | Biomedical Technologies, Stoughton, MA, USA |

Note: GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; mSCF-R: mouse stem cell factor receptor; UCHL1: ubiquitin carboxy-terminal hydrolase L1; NOS1: nitric oxide synthase 1; SNAP25: Synaptosomal-associated protein-25; DLG4: Discs Large MAGUK Scaffold Protein 4; MYH11: myosin heavy chain 11.
### Supplemental Table 3 – Accession Numbers and Primers Used For qPCR

| Target Transcript | Accession numbers | Oligonucleotide sequences (5’-3’) |
|-------------------|-------------------|-----------------------------------|
| GAPDH             | NM_008084.2       | GCCGATGCCCCCATGTTTGTGA (F)        |
|                   |                   | GGGTGGCAGTGATGGCATGGGAC (R)      |
| Ptger1            | NM_013641.2       | GCCTCGTCTGCTCATCCACTCATC (F)     |
|                   |                   | TGGCCAAACCACCAACCAACCAACCAACCA (R) |
| Ptger2            | NM_008964.4       | CTGGATCTTCGAGAGGAGGAAGAGAGA (F)  |
|                   |                   | GCTGGAGGTCCCCACTTTCTTTAGG (R)    |
| Ptger3            | NM_011196.2       | AATCACCCAGGAGACGGACATCCAG (F)    |
|                   |                   | GCAGAAGCCAGGCAACTCCAG (R)       |
| Ptger4            | NM_001136079.1    | CGGGTGCCGAGATCGAGGTGTCAT (F)     |
|                   |                   | GGCCTGCAAATCTGGGTTCGTAAG (R)    |
| Ptgfr             | NM_008966.3       | GTGCAATGGCCGTCAGGAGGAG (F)       |
|                   |                   | TTGTTACCAGAAAGGGACTCCAGCA (R)   |
| Ptgir             | NM_008967.3       | CTCCCCTGCCTTCATGATCCCG (F)       |
|                   |                   | CAGGGGTCCAGGATGGGTGTG (R)       |
| Ptgr1             | NM_025968.3       | CGAGCAAGTGGGCCAGAGTCG (F)        |
|                   |                   | GTCCGGCCACTCTACAGGCA (R)        |
| Hpgd              | NM_008278.2       | TTAGAGGGCTCATGCTGTTGC (F)        |
|                   |                   | TGTGTCACCAAGCTGGGCA (R)         |
| Ptges1            | NM_022415.3       | TGCCCTAGAGCCCACCGCAA (F)         |
|                   |                   | GGAGCGGAAGCGTGGTTCA (R)         |
| Ptgs1             | NM_008969.3       | GCCTAGGCCCACCGGTAGAC (F)         |
|                   |                   | CGCATCAACACGGCGCTGTG (R)        |
| Ptgs2             | NM_011198.3       | GATGCTTTCGAGCTGTGCTG (F)         |
|                   |                   | CATAGAATCCAGTCCGGGTACAGTC (R)   |

Note: GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; Ptger1: prostaglandin E receptor 1 (EP1); Ptger2: prostaglandin E receptor 2 (EP2); Ptger3: prostaglandin E receptor 3 (EP3); Ptger4: prostaglandin E receptor 4; Ptgfr: prostaglandin F receptor (FP); Ptgir: prostaglandin I receptor (IP); Ptgr1: prostaglandin reductase 1; Hpgd: hydroxyprostaglandin dehydrogenase 15-(NAD); Ptges1: microsomal prostaglandin E synthase 1; Ptgs1: prostaglandin-endoperoxide synthase 1 (COX1); Ptgs2: prostaglandin-endoperoxide synthase 2 (COX2). (F): forward primer; (R): reverse primer.
### Supplemental Table 4 – Mean Amplitude and Frequency Values From All 9 Antral Regions in Both Wild-Type and Lep\(^{ob}\) Mice

| Region | Control Amplitude (mV) | Lep\(^{ob}\) Amplitude (mV) | Control Frequency (min\(^{-1}\)) | Lep\(^{ob}\) Frequency (min\(^{-1}\)) |
|--------|-----------------------|-----------------------------|---------------------------------|--------------------------------------|
| 1      | 28.1 ± 4.9 (9)        | 16.7 ± 9.8 (9)              | 3.8 ± 1.0 (8)                   | 5.7 ± 2.0 (9)                        |
| 2      | 28.3 ± 2.0 (8)        | 20.8 ± 8.4 (9)              | 4.0 ± 0.9 (8)                   | 5.4 ± 2.0 (9)                        |
| 3      | 27.2 ± 4.9 (8)        | 23.6 ± 8.0 (9)              | 3.9 ± 1.3 (8)                   | 5.5 ± 1.9 (9)                        |
| 4      | 27.3 ± 6.6 (7)        | 14.0 ± 7.1 (8)              | 3.7 ± 1.0 (8)                   | 5.5 ± 1.6 (8)                        |
| 5      | 30.8 ± 2.6 (9)        | 19.5 ± 6.9 (9)              | 3.5 ± 0.6 (8)                   | 5.2 ± 1.7 (9)                        |
| 6      | 28.0 ± 4.3 (7)        | 21.1 ± 2.9 (8)              | 3.0 ± 0.6 (8)                   | 5.4 ± 2.0 (9)                        |
| 7      | 17.8 ± 9.4 (6)        | 12.0 ± 9.0 (7)              | 2.3 ± 1.3 (6)                   | 4.0 ± 2.6 (7)                        |
| 8      | 21.6 ± 5.2 (7)        | 12.8 ± 8.6 (8)              | 3.0 ± 0.7 (7)                   | 3.8 ± 2.6 (8)                        |
| 9      | 20.1 ± 10.3 (7)       | 14.5 ± 7.1 (6)              | 2.6 ± 0.3 (7)                   | 4.6 ± 1.6 (6)                        |

Note: Values = Mean ± SD; \(n\) numbers in parentheses.
