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Enteroendocrine L Cells Sense LPS after Gut Barrier Injury to Enhance GLP-1 Secretion

Graphical Abstract

Highlights
- LPS induce GLP-1 secretion from L cells through a TLR4-dependent mechanism
- Gut ischemic injury is coupled to immediate GLP-1 secretion in mice and humans
- L cells are mucosal sensors of LPS after gut injury
- GLP-1 secretion is closely related to gut inflammation

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In Brief
Lebrun et al. demonstrate that enteroendocrine L cells sense lipopolysaccharides (pro-inflammatory bacterial compounds) after gut injury and respond by secreting glucagon-like peptide 1. These findings expand concepts of L cell function to include roles as both a nutrient and pathogen sensor, linking glucagon-like peptide secretion to gut inflammation.
Enteroendocrine L Cells Sense LPS after Gut Barrier Injury to Enhance GLP-1 Secretion

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https://doi.org/10.1016/j.celrep.2017.10.008

SUMMARY

Glucagon-like peptide 1 (GLP-1) is a hormone released from enteroendocrine L cells. Although first described as a glucoregulatory incretin hormone, GLP-1 also suppresses inflammation and promotes mucosal integrity. Here, we demonstrate that plasma GLP-1 levels are rapidly increased by lipopolysaccharide (LPS) administration in mice via a Toll-like receptor 4 (TLR4)-dependent mechanism. Experimental manipulation of gut barrier integrity after dextran sodium sulfate treatment, or via ischemia/reperfusion experiments in mice, triggered a rapid rise in circulating GLP-1. This phenomenon was detected prior to measurable changes in inflammatory status and plasma cytokine and LPS levels. In human subjects, LPS administration also induced GLP-1 secretion. Furthermore, GLP-1 levels were rapidly increased following the induction of ischemia in the human intestine. These findings expand traditional concepts of enteroendocrine L cell biology to encompass the sensing of inflammatory stimuli and compromised mucosal integrity, linking glucagon-like peptide secretion to gut inflammation.

INTRODUCTION

Glucagon-like peptide 1 (GLP-1) is secreted from enteroendocrine cells (EECs) and was originally described as an incretin hormone, which also exerts other metabolic actions such as reduction in appetite and food intake (Holst, 2007). These actions supported the development of GLP-1 receptor (GLP-1R) agonists for the treatment of type 2 diabetes. GLP-1 acts through a single receptor widely expressed in multiple tissues, suggesting that it could have additional roles beyond glucose lowering. Notably, GLP-1R agonists exert beneficial effects in experimental preclinical models of inflammatory disease (Lee and Jun, 2016). However, the mechanisms through which incretin therapies control inflammation remain incompletely understood (Drucker, 2016).

The gastrointestinal tract, the site of endogenous GLP-1 production, is colonized by billions of bacteria, some of which produce pro-inflammatory lipopolysaccharides (LPS), which influence gut immunity (Gnauck et al., 2016) and hormone release (Bogunovic et al., 2007). LPS act mainly through the activation of Toll-like receptor 4 (TLR4) (Poltorak et al., 1998). Under normal conditions, several mechanisms restrict LPS within the gut lumen; however, in some situations, especially when intestinal barrier function is compromised, LPS molecules enter the blood circulation and trigger systemic inflammation. Intriguingly, LPS or interleukin-6 (IL-6) increased plasma GLP-1 levels in mice, through incompletely understood mechanisms (Ellingsgaard et al., 2011; Kahles et al., 2014; Nguyen et al., 2014).

Here we show that LPS increase GLP-1 secretion through a TLR4-dependent mechanism. In mice, experimental alteration of the gut barrier led to a rapid rise in plasma GLP-1 levels through mechanisms enabling luminal LPS to access EECs. Remarkably, LPS also stimulated GLP-1 secretion in humans, and GLP-1 levels were rapidly increased in humans with acute experimental intestinal ischemia. Hence, gut barrier alteration facilitates LPS exposure, which in turn augments GLP-1 secretion from local L cells. These findings redefine classical nutrient-stimulated concepts of GLP-1 acting as an...
incretin hormone to include a role for GLP-1-producing L cells as sensors of local inflammation and barrier integrity.

RESULTS

LPS Induce the Expression and Secretion of GLP-1

The actions of LPS to increase circulating GLP-1 (Nguyen et al., 2014) may be mediated by (1) the enhancement of its secretion and/or (2) the inhibition of its degradation. To distinguish between these mechanisms, we investigated the effects of LPS on GLP-1 metabolism in vivo. Mice injected with LPS exhibited marked increases in plasma levels of LPS, measured as 3-hydroxyxymyristate (3-HM) fatty acids (Figure 1A). Intestinal mRNA transcripts encoding pro- and anti-inflammatory cytokines and TLR4 peaked 3 hr after LPS injection (Figures 1B and 1C), and levels of circulating cytokines increased rapidly (Figure 1D). Ideal expression of proglucagon (Gcg, which encodes GLP-1) and prohormone convertase 1/3 (Pcsk1) was rapidly and transiently induced (Figures 1E and 1F). As shown in Figures 1G and 1H, both active and total forms of GLP-1 were induced by LPS injection. Due to ethical concerns and in agreement with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines and reduce, replace, and refine (3R) principles, limited analysis at t = 3 and 6 hr revealed that saline administration had no effect compared to LPS injection (Figure 1I). Furthermore, positive correlations between total GLP-1 and LPS were observed (Figure 1J).

We next determined whether intraluminal LPS could stimulate L cell GLP-1 secretion. Both intraluminal glucose and LPS stimulated GLP-1 secretion from mouse ileum explants (Figure 1K). Furthermore, GLP-1 secretion was modestly but rapidly increased after direct LPS stimulation of GLUTag (Figures 1L and 1M) and STC-1 EECs (Figure 1N). Ex vivo, quantification of intestinal fatty acid-binding protein (I-FABP), a marker of intestinal cell membrane integrity (Pelsers et al., 2005), did not show any differences with the highest concentration of LPS. In vitro, cytotoxicity tests revealed no adverse effects of LPS on the integrity of these two cell lines (data not shown).

LPS Induce GLP-1 Secretion through a TLR4-Dependent Pathway

Although IL-6 has been proposed as the mediator of LPS-induced GLP-1 secretion (Kahles et al., 2014), injection of LPS in IL-6-deficient (Il6−/−) mice (Figure S1A) led to an attenuated but detectable increase in GLP-1 secretion (Figure S1B). Furthermore, LPS did not induce GIP secretion (Figure S1C), making indirect GLP-1 secretion through GIP unlikely. We next blocked muscarinic neural transmission with atropine, which resulted in substantially attenuated basal GLP-1 secretion. In contrast, atropine did not abrogate the stimulatory effect of LPS on GLP-1 secretion (Figure S1D).

Although TLR4 has been considered the classical receptor transducing an inflammatory response to LPS, recent data implicate caspases as a non-canonical inflammasome activation pathway that senses LPS (Hagar et al., 2013). After LPS injection, plasma levels of pro- and anti-inflammatory cytokines were robustly increased in wild-type (WT), but not in TLR4-deficient (Tlr4−/−) mice (Figure 2A); however, the rise in plasma GLP-1 levels was extinguished (Figure 2B), consistent with a critical role for TLR4 in coupling LPS to GLP-1 secretion. As TLR4 is expressed by intestinal EECs (Bogunovic et al., 2007), we next assessed the TLR4 pathway in GLUTag and STC-1 cell lines. GLP-1 secretion was abolished when GLUTag (Figure 2C) or STC-1 cells (Figure 2D) were challenged with LPS in the presence of a TLR4 antagonist, TAK-242 (Figures 2C and 2D). This correlated with an elevation of calcium monitored in single cells in response to LPS in both cell lines (Figures S2A and S2B).

Incubation in calcium-free buffer totally blunted this increase, highlighting the importance of extracellular calcium (Figure S2B). Furthermore, LPS-induced calcium fluxes were blocked by a TLR4 antagonist (Figure S2C). Hence, LPS require TLR4 to induce intracellular calcium flux and GLP-1 secretion in vitro.

Luminal LPS Enhance GLP-1 Secretion after Gut Barrier Injury

LPS administered intravenously (i.v.) or intraperitoneally (i.p.) induced GLP-1 secretion, whereas no effect was observed when LPS were given through an oral gavage (Table S1). These findings imply that LPS may need to reach gut EECs at their basolateral location via the circulation or through luminal exposure in the presence of an altered gut barrier. We next examined animal models in which gut barrier function was compromised. We first studied dextran sodium sulfate (DSS)-induced colitis. As shown in Figure 3A, endotoxemia after an oral administration of LPS in control animals was unchanged over the 6-hr period, yet it increased in DSS-treated mice. Furthermore, plasma GLP-1 levels were increased in DSS-treated mice upon LPS gavage, whereas no increase in GLP-1 was observed in control animals (Figure 3B). Circulating LPS and GLP-1 levels were positively correlated (Figure 3C). Independent of DSS treatment, the oral administration of LPS also led to a marked but transient increase in IL-6 (Figure 3D).

To further assess whether alteration of the gut barrier could facilitate GLP-1 secretion through exposure to endogenous luminal bacterial-derived LPS, we studied mice with mesenteric ischemia/reperfusion (I/R) injury. Gut ultrastructure was highly disorganized after I/R. Short times of I/R were sufficient to damage intestinal villi (Figures S3A and S3B), with further gut injury evident following longer periods of I/R (Figure S3C). Increased GLP-1 levels were observed after a 15-min ischemia/15-min reperfusion period, and they continued to rise after 20-min ischemia/120-min reperfusion period (Figure 3E). Levels of Il6 and Il10 mRNA transcripts were significantly increased with longer times of reperfusion (Figure 3F). Circulating levels of these two cytokines were detectable after 20 min of ischemia and rose with increased reperfusion time (Figure 3G). We next compared levels of GLP-1 and I-FABP. Only GLP-1 levels rose significantly after a short reperfusion time, suggesting that marked epithelial injury is not required for triggering GLP-1 secretion (Figure 3H), whereas plasma levels of LPS, indirectly quantified as 3-HM, were unchanged during the 2 hr of reperfusion (Figure 3I).

To further investigate the role of endogenous luminal LPS, we used two pharmacological approaches to (1) decrease the quantity of active LPS or (2) decrease signaling through the TLR4 receptor. First, we treated mice with Polymyxin B, an antibiotic that binds to and prevents the activity of LPS. In Polymyxin
Figure 1. LPS Enhance GLP-1 Secretion

(A) Plasma 3-HM quantification after LPS i.p. injection in mice (n = 9–10).
(B and C) Ileum cytokines (B) and Tlr4 (C) mRNA relative expression after LPS i.p. injection in mice (n = 9–10).
(D) Cytokine plasma levels after LPS i.p. injection in mice (n = 8).
(E and F) Mouse ileum Gcg (E) and Pcsk1 (F) mRNA relative expression after LPS i.p. injection (n = 9–10).
(G–I) Plasma active GLP-1 (G) and plasma total GLP-1 (H and I) after LPS i.p. injection in mice (LPS injection, n = 9–10; and saline injection, n = 5).
(J) Correlation between plasma 3-HM and total GLP-1.
(K) GLP-1 secreted by mouse ileum explants challenged by glucose or LPS for 1 hr (n = 11–16).
(L) GLP-1 secreted by GLUTag cells after 90 min of glucose or LPS stimulation (n = 6).
(M) Time course of GLP-1 secretion by GLUTag cells after LPS stimulation (n = 4–5).
(N) GLP-1 secreted by STC-1 cells after 90 min of LPS stimulation (n = 4–5).
All results are expressed as mean ± SEM.
B-treated mice subjected to I/R, the induction of GLP-1 secretion was blunted (Figures 3J and 3K). The induction of IL-10 was also slightly reduced (Figure 3L). Second, pretreatment of mice with a TLR4 antagonist attenuated the I/R-induced increase in GLP-1 levels (Figure 3M), associated with a lower GLP-1 secretion index (Figure 3N) and reduced levels of IL-10 (Figure 3O). These results suggest that endogenous luminal LPS induce GLP-1 secretion when gut barrier function is compromised. As intestinal GLP-2 is known to be co-secreted with GLP-1, we tested whether GLP-2 was also increased by LPS or gut injury. Figure S4 shows that LPS injection (Figure S4A) and I/R experiments (Figure S4B) both led to a significant increase in plasma GLP-2 levels.

LPS-Induced GLP-1 Secretion Is Detected in Humans

As shown in Figure 4A, plasma GLP-1 levels were increased 3 hr after LPS, but not saline, injection in human subjects. LPS also induced a transient increase in pro- and anti-inflammatory cytokines (Figure 4B). Finally, we utilized a model of I/R (Grootjans et al., 2010) to evaluate whether I/R injury in the human gut in vivo was associated with an increase in GLP-1 secretion. Arteriovenous differences in plasma GLP-1 levels were measured before, after 45 min of ischemia, and after 30 or 120 min of reperfusion. GLP-1 levels were markedly increased after 45 min of ischemia, and they returned to baseline levels after reperfusion of human intestine (Figure 4C). These results demonstrate that even brief periods of human gut injury are associated with a rapid induction of GLP-1 secretion in vivo.

DISCUSSION

We and others have previously shown that LPS increase plasma levels of GLP-1 in mice (Kahles et al., 2014; Nguyen et al., 2014). Our current study implies that increased secretion of tumor alpha (TNF-α) to the stimulation of GLP-1 secretion (Ellingsgaard et al., 2011; Gagnon et al., 2015).

Under physiological conditions, LPS do not pass through the gut barrier except in small quantities during nutrient ingestion (Erridge et al., 2007). Indeed, as expected, oral administration of purified LPS molecules did not induce an increase in systemic endotoxemia or GLP-1 secretion. However, when gut barrier function was damaged, such as in ileum explants or in DSS-treated mice, exogenous LPS administered into the lumen led to a significant induction of GLP-1 secretion. In the DSS experiment, the increased levels of IL-6 in both groups of mice (at t = 1 hr) might be explained by the activation of B1 cells of the lamina propria (Murakami et al., 1994) and/or by the rapid sensing of bacterial products by intestinal epithelial cells shown to secrete pro-inflammatory cytokines (Kagnoff and Eckmann, 1997).

In pathophysiological situations exemplified by I/R injury, a very rapid secretion of GLP-1 was observed, findings dependent upon the bioavailability of active LPS. However, for longer time periods of reperfusion, GLP-1 release could also be secondary to cell lysis. Nevertheless, we cannot exclude the possibility that additional molecules present in the gut lumen might simultaneously stimulate GLP-1 secretion during gut injury. Notably, under the defined experimental conditions studied herein, we did not observe changes in circulating LPS or in LPS levels in lymph nodes (data not shown). Nevertheless, LPS stimulated GLP-1 secretion when injected into mice or humans. This suggests that LPS could act through a TLR4 receptor located on the basolateral membranes of EECs, consistent with previous findings (Brighton et al., 2015; Vamadevan et al., 2010) and with our observations linking experimental disruption of gut barrier function with stimulation of GLP-1 secretion.

The LPS-enteroendocrine GLP-1 axis described here is consistent with a putative role for EECs in the innate immune response against pathogens, to maintain mucosal immune...
**Figure 1:**

**A** - Graph showing the relationship between 3-HM (ng/mL) and time (hours) for Oral LPS load.

**B** - Graph showing the relationship between Total GLP-1 (pM) and time (hours) for Oral LPS load.

**C** - Graph showing the relationship between IL-6 (pg/mL) and time (hours) for Sham 5 10 15 15 60 120.

**D** - Graph showing the relationship between GLP-1 (pM) and time (hours) for Sham 15 60 120.

**E** - Graph showing the relationship between Total GLP-1 (pM) and time (hours) for Sham 5 10 15 15 60 120.

**F** - Graph showing the relationship between IL-10/Rplp0 mRNA and time (hours) for Sham 5 10 15 15 60 120.

**G** - Graph showing the relationship between IL-6 (pg/mL) and time (hours) for Sham 5 10 15 15 60 120.

**H** - Graph showing the relationship between IL-FABP and time (hours) for Sham 5 10 15 15 60 120.

**I** - Graph showing the relationship between 3-HM (ng/mL) and time (hours) for Sham 5 10 15 15 60 120.

**J** - Graph showing the relationship between Total GLP-1 (pM) and time (hours) for Control and Polymyxin B.

**K** - Graph showing the relationship between Fold (% basal) and time (hours) for GLP-1 and IL-6/IL-10.

**L** - Graph showing the relationship between Fold (% basal) and time (hours) for GLP-1, IL-6, and IL-10.

**M** - Graph showing the relationship between Total GLP-1 (pM) and time (hours) for Control and Antagonist.

**N** - Graph showing the relationship between Fold (% basal) and time (hours) for GLP-1, IL-6, and IL-10.

**O** - Graph showing the relationship between 3-HM (ng/mL) and time (hours) for Control and Polymyxin B.

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homeostasis (Bogunovic et al., 2007; Selleri et al., 2008). Moreover, EECs represent key orchestrators of immune responses to pathogens and commensal bacteria (Worthington, 2015). EECs localized within the mucosa without luminal access may be more easily engaged by LPS molecules after mucosal barrier degradation, as occurs upon I/R injury. The increase in permeability associated with gut injury likely facilitates LPS access to and stimulation of enterocelluar L cells, with resultant GLP-1 secretion. GLP-1 in turn may act as an anti-inflammatory peptide that controls mucosal integrity and attenuates both local and systemic inflammation (Drucker, 2016; Lee and Jun, 2016). Indeed, both Paneth cells (known to produce antimicrobial components) and intestinal intraepithelial lymphocytes have been reported to express a functional GLP-1R (Kedees et al., 2013; Yusta et al., 2015), and loss of the GLP-1R increases the severity of DSS-induced intestinal injury (Yusta et al., 2015).

The physiological relevance of GLP-1 secretion in the control of basal gut barrier homeostasis remains obscure. Nevertheless, lioglulitide, a GLP-1 analog, improved gut permeability in rats (Nozu et al., 2017) and induced barrier protective effect by improving Brunner’s gland function (Bang-Berthelsen et al., 2016). In addition, GLP-1R agonists promoted intestinal growth (Kissow et al., 2012; Simonsen et al., 2007) through fibroblast growth factor 7 (Koehler et al., 2015), and exogenous GLP-1 protects the gut against oxidative damage (Deniz et al., 2015). Whereas the contribution of endogenously secreted GLP-1 to gut growth appears to be modest (Wisemann et al., 2017), endogenous GLP-1 contributes to gut recovery in the pathophysiological context of chemotherapy (Kissow et al., 2013). Thus, the 4.8-fold increase in GLP-1 secretion observed here could also modulate gut homeostasis through local actions to restore gut integrity. L cells also secrete GLP-2, whose role in promoting enterocellular function to include roles as both a nutrient and pathogen sensor. Indeed, recent findings have questioned the relative importance of gut versus pancreatic GLP-1 for the control of glucose homeostasis (Chambers et al., 2017). Our current findings illuminate the importance of GLP-1 in the response to injury and inflammation, expanding concepts of L cell function to include roles as both a nutrient and pathogen sensor.

**EXPERIMENTAL PROCEDURES**

**Animals**

All animal procedures were in accordance with institutional guidelines and approved by the University of Burgundy’s Ethics Committee on the Use of Laboratory Animals (protocol number 5459). All experiments were performed using male C57BL/6J mice (8–12 weeks old); Tlr4−/− mice were provided by Dr. Bernard Ryffel (Hoshino et al., 1999). Blood samples were collected in EDTA-coated tubes. Plasma was separated by centrifugation at 7,200 × g for 10 min at 4 °C. Blood and plasma samples were stored at −20 °C.

**Acute LPS Administration**

LPS were i.p. injected at 1 mg/kg. Details are provided in the Supplemental Experimental Procedures.

**Gut Injury Models**

DSS treatment was adapted from previously described experimental procedures (Kitajima et al., 1999). The oral load of LPS (5 mg/animal) was performed.

**Figure 3. Gut Injury Induces GLP-1 Secretion in Mice**

(A and B) Plasma 3-HM (A) and total GLP-1 (B) levels after LPS gavage in control and DSS-treated mice (n = 8–9).

(C) Plasma 3-HM and total GLP-1 correlation.

(D) IL-6 plasma levels after LPS gavage in control and DSS-treated mice (n = 8).

(E) Total GLP-1 plasma levels after mesenteric I/R (n = 6).

(F) Jejunal cytokine mRNA relative expression after mesenteric I/R (n = 5).

(G) Cytokine plasma levels after 20 min of ischemia and 15, 60, or 120 min of reperfusion (n = 4–5).

(H) Total GLP-1 and I-FABP plasma levels after 20 min of ischemia and 15 or 120 min of reperfusion (n = 6).

(I) LPS blood levels after 20 min of ischemia and 15, 60, or 120 min of reperfusion (n = 5–6).

(J) Total GLP-1 plasma levels before and after I/R (20/15 min) in control and Polymyxin B-treated mice (n = 6).

(K and L) Fold induction of GLP-1 (K) and cytokine (L) plasma levels after I/R (20/15 min) in control and Polymyxin B-treated mice (n = 6).

(M) Total GLP-1 plasma levels before and after I/R (20/15 min) in control and TLR4 antagonist-treated mice (n = 6).

(N and O) Fold induction of GLP-1 (N) and cytokine (O) plasma levels after I/R in control and TLR4 antagonist-treated mice (n = 6).

All results are expressed as mean ± SEM. See also Table S1 and Figures S3 and S4.
after DSS treatment (2.5% [w/v] for 7 days in drinking water). Intestinal I/R was achieved through an occlusion of the superior mesenteric artery. Polymyxin B (0.2 mg/mL for 14 days in drinking water) and TLR4 antagonist (i.p. injection at 0.2 mg/animal) were administered prior to I/R experiments. Details are provided in the Supplemental Experimental Procedures.

**Real-Time qPCR**
Details are provided in the Supplemental Experimental Procedures.

**Human LPS Injection**
Experimental endotoxemia was induced in healthy volunteers (men from 18 to 35 years old) through an i.v. administration of LPS at 2 ng/kg. Experiments were in accordance with the Declaration of Helsinki. After approval from the local ethics committee of the Radboud University Medical Center, volunteers gave written informed consent to participate in the study. Subjects were screened before participation and had a normal physical examination, electrocardiography, and routine laboratory values. Details are provided in the Supplemental Experimental Procedures.

**Human Intestinal I/R**
The experimental protocol was performed as previously described (Grootjans et al., 2010). Experiments were in accordance with the Declaration of Helsinki. The study was approved by the Medical Ethics Committee of the Maastricht University Medical Center, and written informed consent of all patients (six patients with a median age of 67 years, range of 42–85 years) was obtained. Details are provided in the Supplemental Experimental Procedures.

**GLP-1 Secretion Experiments**
Ex vivo GLP-1 secretion was studied in a mice model of ileum explants. In vitro GLP-1 secretion was investigated using the murine enteroendocrine cell lines...
GLUTag and STC-1. Details are provided in the Supplemental Experimental Procedures.

Biochemical Analysis
LPS concentrations were measured through the quantification of 3-HM levels according to the procedure previously described (Pais de Barros et al., 2015). Active/total GLP-1 and I-FABP concentrations were determined by commercially available ELISA Kits (EGLP-35K, Millipore; EZGLP1T-36K, Millipore; E-EL-M0735, Elabscience) in accordance with the manufacturers’ protocols. Cytokine plasma levels were measured by Milliplex MAP 5-Plex Kit using mouse cytokine/chemokine magnetic bead panel (MCYTOMAG-70K, Millipore), according to the manufacturer’s protocol, and using a LumineXr apparatus (Bio-Plex 200, Bio-Rad).

Statistical Analysis
Data are presented as mean ± SEM. Statistical analyses were performed using Prism (GraphPad). To decide whether to use parametric or non-parametric statistics, the normality of distributions was assessed with the Shapiro-Wilk test (under n = 7, distributions were considered to be non-normal). Statistical significance of differences between two groups was evaluated with the Mann-Whitney U test or the Sudent’s t test (a statistical correction was applied when variances were different between groups). For more than two groups, Kruskal-Wallis with Dunn’s post hoc test or one-way ANOVA with Dunnett’s post hoc test was performed. Spearman correlations were calculated between groups. A value of p < 0.05 was considered statistically significant (NS, not significant; *p < 0.05, **p < 0.01, and ***p < 0.001).

SUPPLEMENTAL INFORMATION
Supplemental Information includes Supplemental Experimental Procedures, four figures, and one table and can be found with this article online at https://doi.org/10.1016/j.celrep.2017.10.008.

AUTHOR CONTRIBUTIONS

ACKNOWLEDGMENTS
This work was supported by the INSERM, Centre de Recherches UMR 1231, the University Bourgogne Franche-Comté, a French government grant managed by the French National Research Agency under the program “Investissements d’Avenir” with reference ANR-11 LABX-0021, the FEDER, and the Regional Council of Bourgogne Franche-Comté. D.J.D. is supported by the Canada Research Chairs program, CIHR grant 154321, and the Banff & Best Diabetes Centre-Novo Nordisk Chair in Incretin Biology. The authors gratefully acknowledge A. Hamman and S. Monier from the platform of Cytometry; E. Charron, H. Choubley, and V. Bergas from the Lipidomic platform; A. Chêlémare, A. Geissler, and A. Bouchot from the platform of Histology; and V. Saint-Giorgio from the Centre de Zootechnie of the Université de Bourgogne for animal care.

Received: March 29, 2017
Revised: July 25, 2017
Accepted: October 2, 2017
Published: October 31, 2017

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