SIRT1 inhibits the mouse intestinal motility and epithelial proliferation

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1USDA/ARS Children’s Nutrition Research Center, Department of Pediatrics, and 4Division of Diabetes, Endocrinology and Metabolism, Department of Medicine, Baylor College of Medicine, Houston; 2Department of Animal Science, Shandong Agricultural University, Shandong, China; 3Departments of Integrative Biology and Pharmacology, the University of Texas Medical School, Houston, Texas

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Wang Y, Shi X, Qi J, Li X, Uray K, Guan X. SIRT1 inhibits the mouse intestinal motility and epithelial proliferation. Am J Physiol Gastrointest Liver Physiol 302: G207–G217, 2012. First published October 28, 2011; doi:10.1152/ajpgi.00302.2011.—Sirtuin 1 (SIRT1), a NAD+-dependent histone deacetylase, is involved in a wide array of cellular processes including glucose homeostasis, energy metabolism, proliferation and apoptosis, and immune response. However, it is unknown whether SIRT1 plays any physiologic role in the regulation of intestinal homeostasis and motility. Thus the aim was to define SIRT1 expression and function in the gastrointestinal (GI) tract under physiological conditions. Forty 12–14-wk-old SIRT1 knockout (KO) and wild-type (WT) mice were fasted 21 h and/or refed 3 h. Fasted mice were injected intraperitoneally with bromodeoxyuridine (120 mg/kg body wt) 2 h before euthanasia. SIRT1 protein was localized to gastric and intestinal epithelial nuclei and was responsive to the nutritional status. SIRT1 was required for intestinal epithelial homeostasis. The SIRT1 KO mice showed enhanced crypt proliferation and suppressed villous apoptosis, resulting in increased intestinal villous height. In the SIRT1 KO intestine, the abundance of Forkhead box protein O1 and p53 protein decreased, whereas the subcellular localization of β-catenin protein accumulated mainly in the crypts. The SIRT1 KO mice showed accelerated gastric emptying rate with increased abundance of ghrelin mRNA and protein in the stomach. Moreover, the SIRT1 KO mouse intestine showed enhanced ex vivo spontaneous contraction. We concluded that, SIRT1 plays a critical role in the control of intestinal homeostasis (by promoting apoptosis and inhibiting proliferation) and gastrointestinal motility (by reducing gastric emptying and intestinal contractile activity), implicating a novel role for SIRT1.

sirtuin 1: epithelial homeostasis; intestinal contraction; gastric emptying; ghrelin

THE NAD+-DEPENDENT DEACYLASE sirtuin 1 (SIRT1) is involved in the regulation of diverse biological processes including cell proliferation, differentiation, and apoptosis, as well as glucose homeostasis and energy metabolism, insulin secretion and sensitivity, and immune response. SIRT1 silences gene transcription by deacetylating histone and nonhistone proteins (2). Although SIRT1 function has been intensively investigated in the brain and peripheral tissues, its physiological relevance remains largely unknown in the gastrointestinal (GI) tract, which plays a key role in the control of energy homeostasis.

As the major contributor of energy, glucose uptake by the gut is influenced by gastric emptying (21). Gastric emptying is fine-tuned by metabolic, neuronal, and hormonal signals. Gastric emptying may serve as a key factor in the control of energy homeostasis (8). Circadian rhythms govern a variety of metabolic and physiological functions (27, 33), which is controlled by the circadian clock. SIRT1 is a component of CLOCK-BMAL1 transcription complex and acts as a negative regulator of the clock gene expression (e.g., Per2, a key repressor of the circadian clock) (26). In addition, SIRT1 can regulate clock gene expression through deacetylation and degradation of Per2 (1) and is required for high-magnitude circadian rhythms underlying fine-tuning gastric emptying and intestinal contraction (11, 29). Thus we wanted to test whether SIRT1 deficiency in mice impairs the GI motility.

It is controversial whether SIRT1 inhibits apoptosis and promotes survival in epithelial cells. In fact, SIRT1 has shown both oncogenic and tumor-suppressive effects (19). SIRT1-mediated suppression of apoptosis depends on the status of tumor suppressor p53 acetylation in a tissue-specific manner (2, 4, 25). SIRT1 directly deacetylates and inactivates p53, downregulating p53-mediated growth arrest and apoptosis (7, 20, 35). On the other hand, however, depletion of SIRT1 in mice increases genomic instability, suggesting that SIRT1 acts as a tumor suppressor (7). Importantly, ectopic induction of SIRT1 suppresses intestinal tumorigenesis in vivo (9). SIRT1 deacetylates β-catenin, promotes cytoplasmic localization, and suppresses its ability to activate transcription and to drive cell proliferation (9). Moreover, inhibition of SIRT1 stimulates proliferation of human colon cancer HCT116 cells, indicating that SIRT1 is an inhibitor of proliferation and tumor formation in colon cancer (14). However, the physiological role of SIRT1 in intestinal homeostasis has not been defined. Intestinal homeostasis is maintained through a rapid turnover of cell proliferation and apoptosis. Cell proliferation in the crypt is enhanced upon feeding but decreased during fasting. In contrast, cell apoptosis along the villus is increased during fast but decreased upon feeding. Thus the intestinal epithelial crypt-villus unit might be an excellent model for defining the physiological role of SIRT1 in the regulation of proliferation and apoptosis simultaneously in vivo. In this report, we showed that the SIRT1 protein was exclusively expressed in the GI epithelial nuclei. As a key cellular energy sensor, the protein abundance of SIRT1 in the stomach and gut is increased during fast. We hypothesized that SIRT1 regulates proliferation and apoptosis of the gut epithelium in response to energy availability, which might underlie nutrition-mediated epithelial homeostasis.

MATERIALS AND METHODS

Experimental procedures. All experiments were approved by the Institutional Animal Care and Use Committee of Baylor College of Medicine. The SIRT1 knockout (KO) mice were provided by Dr. Wenyong Chen (City of Hope’s Beckman Research Institute, Duarte,
CA) upon an agreement of Dr. Frederick Alt (Children’s Hospital, Harvard Medical School, Boston, MA). The mice were weaned at ~6 wk after birth, provided free access to water and a standard rodent diet (no. 2920; Harlan Laboratories, Indianapolis, IN), and maintained under the standard 12-h/12-h light/dark cycle. The mice were genotyped using primers (forward: 5’-CTT GCC CTT CAA GGG ACC AA-3’; reverse 1: 5’-GTA TAC CCA CCA CAT CTG AG-3’; and reverse 2: 5’-CTA CTC CTC CTG GCT ACC AA-3’). Homozygous male mice (SIRT1+/− = KO; SIRT1+/+ = wild-type, WT) were used in the study. For the fast-refeeding experiment, mice at the age of ~12–14 wk were fasted for 21 h and/or then refed for 3 h (n = 10 each group). Mice were injected intraperitoneally with bromodeoxyuridine (BrdU) (Sigma-Aldrich, St. Louis, MO) at a dosage of 120 mg/kg body wt 2 h before euthanasia under isoflurane anesthesia. The mouse intestine and stomach were removed and flushed with ice-cold saline. The intestine (excluding the duodenum) was divided into two equal portions (proximal = jejunum, and distal = ileum). The tissue samples were snap-frozen immediately in liquid nitrogen and stored at −80°C for molecular analysis, or fixed in 10% neutral buffered formalin and embedded in paraffin for morphometry and immunohistochemistry.

Morphometry, immunohistochemistry, and BrdU labeling. One set of paraffin sections was cut at 7.5 μm, deparaffinized, rehydrated, and stained with hematoxylin and eosin for morphometry. Jejunum villus height and crypt depth were quantified under an Axiohot microscope (Zeiss, Jena, Germany) by a blinded examiner using the NIH image software (v1.60) in at least 20 vertically well-oriented villus-crypt units each sample.

Another set of paraffin sections were then microwaved for 20 min in 10 mM citrate buffer (pH 6.0) for antigen retrieval and permeabilized in 0.1% Triton X-100 in PBS (PBS-T) for 3 × 15 min. After being blocked for 1 h (in 10% normal donkey serum in PBS-T), sections were incubated at 4°C overnight with primary antibodies (1:100); antibodies against β-catenin and cleaved caspase 3 from Cell Signaling Technology, Danvers, MA; antibody against ghrelin from Santa Cruz Biotechnology, Santa Cruz, CA; antibody against SIRT1 from Millipore, Billerica, MA; or nonspecific isotype IgG as negative control). Note that the ghrelin antibody recognizes both acyl ghrelin and des-acyl ghrelin. After washing, sections were incubated for 2 h with FITC-conjugated donkey anti-mouse or anti-rabbit IgG (1:1,000; Jackson ImmunoResearch Laboratories, Bar Harbor, ME). Meanwhile, BrdU antibody conjugated with FITC (1:100; BD Bioscience, San Jose, CA) was used to directly stain BrdU-positive labeled cells. The nuclei were counterstained with TOPRO-3 (Invitrogen, Carlsbad, CA). The sections were mounted with 30% glycerol in PBS and visualized via a laser confocal microscopy. Finally, the proportion of proliferating crypt cells was quantified by counting the number of BrdU-labeled nuclei in at least 20 vertically well-oriented crypts in each sample and expressing this as a percentage of total nuclei per crypt. In addition, fractional distribution of ghrelin-positive cells was estimated by immunohistochemistry and counted per image.

Western blotting. Proteins were extracted from the mouse gut. Tissue samples were powdered in liquid nitrogen and homogenized and sonicated on ice in RIPA buffer (50 mM Tris·HCl at pH 7.4, 1% Triton X-100, 0.25% Na-deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 1 mM sodium orthovanadate, 1 mM sodium fluoride) and centrifuged at 10,000 × g for molecular analysis, or fixed in 10% neutral buffered formalin and embedded in paraffin for Western blotting.

SDS-PAGE gels were transferred to methanol presoaked PVDF membrane at 100 V, 4°C for 60 min. After being blocked in PBS-T containing 5% milk, the membrane was incubated at 4°C overnight with primary antibodies 1:500; against cleaved caspase 3 and p53 from Cell Signaling Technology; against SIRT1 from Millipore; and against Forkhead box protein O1 (FoxO1) and β-actin from Santa Cruz Biotechnology. After being washed, the membrane was incubated with anti-rabbit (or mouse) IgG-conjugated horseradish-peroxidase (1:3,000; Bio-Rad Laboratories, Hercules, CA) and reacted with ECL-Plus chemiluminescent reagent (Amersham Biosciences, Piscataway, NJ). Image on the membrane was captured by ChemiDoc XRS and analyzed with Quantity One 4.6.6 (Bio-Rad Laboratories). Finally, the densitometry of protein abundance was normalized by the loading control (namely β-actin).

qRT-PCR. Total RNA was extracted from the frozen tissue samples using RNeasy Mini kit (Qiagen, Valencia, CA). The RNA concentration was determined by NanoDrop 1000 (NanoDrop, Wilmington, DE). Reverse transcription was performed with 1 μg RNA, random hexamers, and oligo (dT)12-18 using SuperScript-III-First-Strand-Synthesis- SuperMix kit (Invitrogen). mRNA abundance was quantified by real-time qRT-PCR using SYBR Green I dye. Forward and reverse primers were 5′-TCCAAGAAGCCACCACTAAGA and 5′-AA-CATCGAAAGGAGCATTGGA for ghrelin (NM_021488); 5′-ATGTCGCCCATCACAAGA and 5′-CCGAAGCTAATGTCGCTG for SIRT1 (NM_021488); and 5′-GCAGACGTCAAGGC-GAGAT and 5′-GCCCTTTCCATGGTGGTGAA for Gapdh (NM_008084.2). Assays were performed in triplicate with an ABI 7900HT Fast real-time PCR system (Applied Biosystems, Foster City, CA). qPCR conditions were as follows: 10 min at 95°C, then 40 cycles of 30 s at 95°C and 60 s at 60°C. Data were normalized to Gapdh or 18S ribosomal RNA (ΔΔCt analysis) as indicated.

13C-octanoic acid breath test. Mice (~12 wk old) were fasted overnight for assessing gastric emptying rate (GER) using 13C-octanoic acid breath test (30), which was modified and coupled with real-time measurement of CO2 production using the Comprehensive Laboratory Animal monitoring system. In brief, mice were transferred and adapted into the calorimetric chamber for 1 h before gastric administration of 13C-octanoic acid-containing meal. To minimize effects of circadian rhythm and gastric volume on the GER, mice were tested between 9:00 and 11:00 AM. A baseline breath sample was taken before the gastric tracer administration. The test meal was composed of 5% (vol/vol) 13C-octanoic acid (99% enrichment; Cambridge Isotope Laboratories, Andover, MA) in milk, which produced a mean initial 13C enrichment of breath CO2. This test meal was administered at 8 μl/g body wt into the stomach via orogastric gavage. After the gastric administration, breath CO2 was sampled every 3.5 min from the chamber and injected into evacuated 10-ml Exetainer tubes for 15 s. The 13C enrichment of breath samples was measured by an isotope ratio mass spectrometry (Thermo Finnigan Deltaplus XL, San Jose, CA) and expressed as atom percentage. The measured 13CO2 recovery in the breath was expressed as percentage excretion per hour of the given 13C-octanoic acid dose (% dose/h). Data for the 13CO2 excretion rate were fitted by a linear regression model (10) using the NLIN Procedure (SAS Version 9.1; SAS Institute, Cary, NC): y = a + e−c×t, where y is the percentage of 13CO2 excretion in breath per hour, t is time in hours, and a, b, and c are regression-estimated constants. The following parameters were computed (15): half-excretion time (T1/2, min) = gamma inv (0.5, b+1, 1/c); lag phase (Tlag, min) = b/c; and gastric emptying coefficient = In (a).

Intestinal contractile activity. To measure intestinal contractile activity (34), strips (~10 mm in length from the distal ileum) were mounted in 5-ml organ baths filled with Krebs-Ringer solution (103 mM NaCl, 4.7 mM KCl, 1.1 mM KH2PO4, 1.1 mM NaHCO3, and 15 mM glucose) that was buffer with BSA and gassed with 5% CO2-95% O2. Isometric force was monitored by an isometric force displacement transducer (Grass FT.03; Grass Instrument, Quincy, MA) connected to a PowerLab (AD Instruments, Colorado Springs, CO). A 0.4-g load was applied to the 1-cm ileal strip when it was first mounted. After a 30-min equilibration, spontaneous contractile activity was recorded under this resting load of 0.4
g, without further imposed stretch. Contractile activity was calculated as the area under the curve integrated for 10 min and/or normalized by the tissue weight.

Statistical analysis. Data were analyzed by the MIXED Procedure (SAS Version 9.1), in which fixed effects (including genotype, treatment, and time), random effects (including individual mouse), and repeated measures (at different time points in the same mouse) were considered. A full model for ANOVA was employed including genotype, treatment, time, and their interactions. If applicable, repeated measures (such as data for gastric emptying) were included in a repeated statement. Data were expressed as means ± SE. P < 0.05 or 0.01 was considered as statistical significance.

RESULTS

SIRT1 deficiency accelerated gastric emptying and increased intestinal contraction. Although SIRT1 is highly expressed in the brain and peripheral tissues (including liver and pancreas), it is unknown whether SIRT1 is expressed in the stomach or gut. To explore the physiological role of SIRT1, we determined

![Fig. 1. Sirtuin 1 (SIRT1) protein expressed in the gastric epithelium.](image)

Fig. 1. Sirtuin 1 (SIRT1) protein expressed in the gastric epithelium. −13–14-wk-old male mice were fasted for 21 h and/or then refed for 3 h. The SIRT1 protein (in green) was mainly localized to the epithelial nuclei, where nuclei were counterstained with TOPRO-3 (in blue). KO, knockout. WT, wild-type.
its cellular localization in the GI tract. SIRT1 protein (in green) was mainly localized to the gastric mucosal epithelium (Fig. 1). Notably, SIRT1 in the WT stomach was induced during fasting and distributed in the cytoplasm and nucleus. However, SIRT1 was less abundant and mainly localized in the nuclei during refeeding. Of note, we observed that the stomach was empty at 1 h after refeeding in the KO mice, whereas it was full in the WT mice.

Whole-body energy balance can be determined more precisely by net intake of food energy and expenditure of body energy. Apparently, digestion and absorption of food affect net intake of food energy, which is mainly regulated by gastric emptying and intestinal motility. We hypothesized that SIRT1 might function as an energy sensor in the GI tract and modulate its motility to control net intake (input) of food energy. Thus we wanted to quantify the rate of gastric emptying in the KO mice using $^{13}$C-octanoic acid breath test. It is well established that gastric emptying is a limiting step for and positively correlated with the rate of $^{13}$CO$_2$ release into breath (from oxidation of $^{13}$C-octanoic acid in the liver). Gastric emptying

![Figure 2](http://ajpgi.physiology.org/) Gastric emptying and intestinal contraction accelerated in SIRT1 KO mice. Rates of gastric emptying (indicated by the rate of $^{13}$CO$_2$ recovered in breath) were higher during the first 30 min in the SIRT1 KO mice than those in the WT littermates (A). The KO mice had shorter half-excretion time and lag phase but higher gastric emptying coefficient than the WT mice (B–D). Representative traces of the spontaneous contractions from ileum strips are shown in E. Due to enhanced amplitude and sustained frequency, the ex vivo spontaneous contraction activity of the ileum increased in the SIRT1 KO mice compared with that in the WT littermates (F–H). Male mice (~12 wk-old) were fasted overnight for assessing gastric emptying rate of the test milk using $^{13}$C-octanoic acid breath test in conjunction with real-time measurement of CO$_2$ production. Data for the $^{13}$CO$_2$ excretion rate were fitted by a nonlinear regression model (10) (see computation in MATERIALS AND METHODS). Spontaneous contraction of the gut was traced ex vivo by isometric force measurement (E). Data are expressed as means ± SE (n = 10 per group). *P < 0.05, **P < 0.01 denote significance (at the same time) between 2 genotypes. AUC, area under the curve.
Fig. 3. The abundance of ghrelin mRNA and protein increased in the SIRT1 KO mouse stomach. The ghrelin protein was expressed in the gastric oxyntic glands (A). Not only the abundance of ghrelin mRNA but also the number of ghrelin-positive cells were higher in the gastric mucosa in the SIRT1 KO mice than those in the WT littermates (B). Interestingly, the abundance of Per2 mRNA increased in the KO stomach during fasting (B). 13–14-wk-old male mice were fasted for 21 h and/or then refed for 3 h. The ghrelin protein (in red) was localized to endocrine cells, where nuclei were counterstained with TOPRO-3 (in blue). Gene expression was measured by real-time qRT-PCR and expressed in terms of mRNA levels relative to 18S rRNA. Data are expressed as means ± SE (n = 10 per group); **P < 0.01 denotes significance in the same genotype between fast and refeeding, whereas a,bP < 0.05 denotes significance during fast (or refeeding) between 2 genotypes.
SIRT1 KO IMPAIRS GI MOTILITY AND HOMEOSTASIS

SIRT1 deficiency increased the abundance of ghrelin mRNA but decreased that of Per2 mRNA in the stomach. Ghrelin significantly stimulates GI motility and inversely associates with expression of circadian genes (namely Per1 and Per2). In response to energy availability, the SIRT1 represses expression (indicated by the rate of $^{13}$CO$_2$ recovered in breath) increased during the first 30 min in the KO mice (Fig. 2A). To derive key parameters for gastric emptying, the data were further fitted by a nonlinear regression model. Both half-excretion time and lag phase for liquid meal were shorter, whereas gastric emptying coefficient was higher in the KO mice than those in the WT mice (Fig. 2, B–D). Importantly, moreover, the ex vivo spontaneous contractile activity of the KO mouse gut increased as measured by isometric force (Fig. 2, E–H). Figure 2E shows representative traces of the spontaneous contractions from the mouse ileum strips, and Fig. 2, F and G, show statistical results of the frequency duration and amplitude of spontaneous contraction. Note that frequencies of spontaneous contractions were approximately at 0.5 Hz (= 30 times/min) and not altered between the WT and KO mice. This is consistent with a notion that the mouse ileum contracts at a very regular frequency of ~30 times per minute. However, the amplitude of spontaneous contraction was enhanced in the KO mice compared with that in the WT. Therefore, the KO mice show higher spontaneous contractile activity (i.e., integral area under the curve in Fig. 2H) attributed to enhanced amplitude. These data indicated that gastric emptying and gut contraction were accelerated in the SIRT1 KO mice.

Fig. 4. SIRT1 protein expressed in the intestinal epithelial nuclei. ~13–14-wk-old male mice were fasted for 21 h and/or then refed for 3 h. The SIRT1 protein (in green) was mainly localized to the epithelial nuclei, where nuclei were counterstained with TOPRO-3 (in red).
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of circadian genes and modulates clock outputs in the control of energy metabolism. Thus we wanted to explore whether SIRT1 deficiency affects expression of ghrelin and Per2 in the stomach (Fig. 3). Representative images for ghrelin-immuno-stained cells (in red) in the stomach are shown in Fig. 3A. The number of ghrelin-positive cells in the gastric epithelium was higher ($P < 0.01$) in the KO mice than that in the WT mice (Fig. 3B). The mRNA abundance of ghrelin precursor in the stomach was higher ($P < 0.01$) during fasting in the KO mice than that in the WT mice but was insignificant during the 3-h refeeding. Of note, the mRNA abundance of ghrelin precursor was not decreased by 3-h refeeding. The expression of ghrelin in the stomach is negatively correlated to that of Per2, a key circadian gene that is negatively modulated by SIRT1. The mRNA abundance of Per2 in the stomach increased ($P < 0.01$) under the fast status in the KO mice compared with that in the WT mice. Moreover, it was lower ($P < 0.01$) under the fast status than that under the refeeding status in the WT mice, but this response was abolished in the KO mice.

**SIRT1 deficiency stimulates cell proliferation in the gut.** We further determined SIRT1 protein expression and localization in the gut (Fig. 4). Similarly, SIRT1 protein expression was responsive to the nutritional switching from fasting to refeeding. SIRT1 was expressed in the epithelium and exclusively localized to the nuclei. We wanted to define whether SIRT1 plays a physiological role in the maintenance of intestinal homeostasis. Using in vivo 2-h BrdU pulse-chase labeling, we found that BrdU-labeled cells (in green in Fig. 5) were mainly localized to the crypt, a highly proliferating zone, and at a higher percentage (of crypt epithelial cells, $P < 0.01$) in the SIRT1 KO mouse gut, indicating that SIRT1 might suppress intestinal cell proliferation under a normal, physiological state. Because SIRT1 gain-of-function suppresses β-catenin signaling in the gut, we wanted to express whether SIRT1 physiologically inhibits β-catenin signaling underlying suppression of cell proliferation. We found that the total β-catenin was expressed along the entire mucosal crypt-villous unit and mainly localized to the cytoplasm (and plasma membrane) in the WT mice (Fig. 6). However, β-catenin was highly preserved in the crypt and mainly localized to the nucleus (and cytoplasm) in the KO mice. This cellular distribution of β-catenin might indicate that SIRT1 regulates β-catenin shuttling between the cytoplasm and nucleus or β-catenin gene expression and protein turnover per se.

**SIRT1 deficiency suppresses cell apoptosis in the gut.** Intestinal epithelial homeostasis is determined by proliferation and cell death (mainly apoptosis under physiological conditions). We wanted to define whether SIRT1 physiologically modulates intestinal epithelial apoptosis. We found that SIRT1 deficiency suppressed proapoptotic signaling in the gut (Fig. 7). Clearly, active caspase 3 (in green, a marker for cells undergoing apoptosis) was mainly expressed in the villous epithelial cytoplasm. Notably, active caspase 3 protein was less abundant in the SIRT1 KO gut during fasting. These results suggest that SIRT1 might promote the intestinal epithelial cell apoptosis under fasting. Furthermore, we found that protein abundance of transcription factor FoxO1 and tumor suppressor p53 in the gut, which are downstream targets of SIRT1 and key positive regulators of cell apoptosis, was decreased in the KO mice compared with that in the WT mice. Clearly, protein abundance of FoxO1 and p53 in the WT gut was increased under the fast status compared with that under the refed status. But this was not the case in the KO gut. Except for posttranslational modification (i.e., deacetylation), SIRT1 might suppress its degradation by ubiquitination and/or enhance its expression per se. As a result of SIRT1-mediated suppression of proliferation and promotion of apoptosis, villous height was increased in the SIRT1 KO jejunum although crypt depth was not different (Fig. 8).

**DISCUSSION**

SIRT1 is induced to adjust energy metabolism in response to caloric restriction and probably mediates mammalian longevity. The NAD$^+$-dependent SIRT1 plays a pivotal role in metabolic control in response to energy availability (28). The GI integrity and function depend on homeostatic control of cell proliferation and apoptosis in response to luminal nutrition, microbiome, injury, or inflammation. SIRT1, a NAD$^+$-dependent class III histone deacetylase, is involved in a wide array of cellular processes including energy metabolism, proliferation and apoptosis, and immune response. Although energy homeostasis has been focused on energy expenditure and food intake, dietary net energy varies with wide-ranging efficiency rates of digestion and absorption, which is determined by intestinal motility and absorptive surface. However, it is unknown whether SIRT1 plays a physiological role in the regulation of...
intestinal homeostasis. Thus we wanted to test whether energy-sensitive SIRT1 promotes the whole-body energy homeostasis at the first pass by fine-tuning the intestinal motility and homeostasis. In the present study, we showed that SIRT1-null mice had accelerated gastric emptying and gut contraction in conjunction with increased abundance of ghrelin mRNA and protein in the stomach. Moreover, the intestinal homeostasis was disrupted in the SIRT1-null mice, resulting in increased cell proliferation and decreased apoptosis. Furthermore, fast-induced proapoptotic signaling in the gut was abolished in the SIRT1-null mice. Therefore, the energy-sensitive SIRT1 deacetylase finely tunes gastric emptying and intestinal mucosal homeostasis in response to the nutritional status.

Gastric emptying is fine-tuned by metabolic, neuronal, and hormonal signals. In fact, gastric emptying may serve as a key factor in the control of energy homeostasis. In the present study, $^{13}$C-octanoic acid was used in liquid milk for breath test. Of note, $^{13}$CO$_2$ excretion in the mouse breath test is insignificant after 60 min for liquid meal, but it is measurable until 180 min for solid meal (32). Half-excretion time ($T_{1/2} = 29$ min) in the WT mice was similar as reported $T_{1/2} (= 28$ min) for the mouse gastric emptying of liquid meal (32). The $T_{1/2}$ in the KO mice was shorter than that in the WT, suggesting that SIRT1 deficiency accelerates GER of liquid meal. However, it would be interesting to determine whether there is any difference in gastric emptying of solid meal. Interestingly, we noticed that the SIRT1 KO mice had higher food intake per unit of body weight in the present study, which might result from accelerated gastric emptying. In the present study, SIRT1 deficiency accelerated GER and rate of intestinal contraction and increased the abundance of ghrelin mRNA and protein in the stomach. Ghrelin is produced predominantly by the X/A cells within the gastric mucosa (6). Ghrelin mediates gastric phase III-like contractions, enhances gastric emptying, and stimulates small intestinal transit (3). Importantly, the gastric abundance of ghrelin mRNA and protein was higher in the SIRT1 KO mice, and this is associated with accelerated gastric emptying and intestinal motor activity. It is demonstrated that there are two subpopulations of ghrelin cells in the stomach. Whereas acyl ghrelin- and des-acyl ghrelin-immunopositive reactions overlap in closed-type round cells, des-acyl ghrelin-immunopositive reaction occurs in open-type cells where acyl ghrelin is immunonegative (22). The ghrelin antibody (used in the present study) recognizes both acyl ghrelin and des-acyl ghrelin. Thus further studies are warranted to define which form (acyl vs. des-acyl) ghrelin is regulated by SIRT1. Ghrelin accelerates gastric emptying mainly via activation of the vagus and to some extent by activation of peripheral receptors on enteric neurons but not on smooth muscle. It is unlikely that the SIRT1 deficiency-induced enhancement in the ex vivo sponta-
neous contractility of the ileal strip is mediated via a direct action of ghrelin. However, further studies are warranted to determine whether ghrelin is the key mediator for SIRT1 action on gastric emptying and intestinal contraction. For example, it would be interesting to determine whether specific antagonists of ghrelin receptor can block SIRT1 deficiency-enhanced gastric emptying and gastric contractility.

The transcriptional activator clock is a histone acetyl transferase, whereas the NAD$^+$-dependent SIRT1 functions as a histone deacetylase, which links the circadian clock with energy metabolism (23, 27). SIRT1 enhances the deacetylation and degradation of Per2 (1). Both SIRT1 gain-of-function and SIRT1 activators repress the Per2 promoter activity, whereas SIRT1 inhibitors have the opposite effect. Ghrelin and Per2 are rhythmically but inversely expressed in stomach X/A cells and synchronized by food-entrainable circadian clock (16). Without little effect of SIRT1-mediated repression, abundance of ghrelin mRNA and protein increased in the KO mouse stomach. Under the fast status, SIRT1-mediated repression of Per2 expression was alleviated in the SIRT1 KO mouse stomach and hypothalamus (Fig. 9). Interestingly, the abundance of Per2 mRNA in the hypothalamus was increased under the SIRT1 deficiency. Thus SIRT1 deficiency might enhance the circadian oscillation in the stomach and the circadian clock in the hypothalamus, probably amplifying circadian rhythm of gastric emptying and gut contraction. In the present study, however, we cannot conclude whether Per2 directly regulates ghrelin expression and signaling and whether ghrelin is the key mediator for SIRT1-induced action on gastric emptying and intestinal contraction. Further studies are warranted to elucidate how SIRT1 mediates gastric emptying and intestinal homeostasis. For example, it would be important 1) to define whether Per2 directly regulates ghrelin expression using ghrelin promoter-driven luciferase assay, 2) to determine whether ghrelin is the key mediator for SIRT1-induced action on gastric emptying using tissue-specific deletion or overexpression of SIRT1 mouse models.

SIRT1 acts as an energy-sensitive growth suppressor in certain cell types (24). SIRT1-mediated deacetylation of substrates (namely p53 and β-catenin) is involved in the regulation of cell proliferation and apoptosis. However, it is unknown whether SIRT1 plays a physiological role in the control of...
Inhibition of SIRT1 inactivates promoting cell proliferation of the crypt epithelium (14, 18). Wnt/β-catenin signaling plays a crucial role in driving epithelial transcription and proliferation (9). In the present study, β-catenin was accumulated in the KO intestinal crypts, which might account for higher proliferation, supporting the notion that SIRT1 deacetylates β-catenin and reduces transcriptional activity, thus suppressing proliferation in vivo. In this present study, we show that SIRT1 signaling was responsive to the nutritional status and essential for the intestinal epithelial homeostasis. However, further studies are warranted to dissect molecular mechanisms underlying SIRT1-mediated modulation of intestinal homeostasis in response to nutrition.

In summary, we demonstrate that SIRT1 inhibits gastric emptying and intestinal contraction, which is associated with dysregulated expression of Per2 and ghrelin in the stomach; SIRT1 suppresses cell proliferation with unstabilizing cytosolic β-catenin in the intestinal crypts; and SIRT1 promotes cell apoptosis with enhancing p53 function in the intestinal villi. Our study suggests that SIRT1 activation is essential for the circadian control of gastric emptying and intestinal contraction, and for the nutritional modulation of intestinal mucosal homeostasis.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Y. Wang, X. Shi, J. Qi, X. Li, and X. Guan contributed to the study concept and design; acquisition of data; statistical analysis; interpretation of data; and drafting the manuscript. In addition, X. Guan obtained funding, supervised the study, and finalized the manuscript. K. Uray contributed to acquisition, analysis, and interpretation of ex vivo contraction data; and critical revision of the manuscript.

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