Biochemical and Biophysical Research Communications 513 (2019) 974–982

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Inhibition of GIP signaling extends lifespan without caloric restriction

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ARTICLE INFO

Article history:
Received 27 March 2019
Accepted 4 April 2019
Available online 17 April 2019

Keywords:
Adipose tissue
Gene expression
Mouse

ABSTRACT

Aims/introduction: Caloric restriction (CR) promotes longevity and exerts anti-aging effects by increasing Sirtuin production and activation. Gastric inhibitory polypeptide (GIP), a gastrointestinal peptide hormone, exerts various effects on pancreatic β-cells and extra-pancreatic tissues. GIP promotes glucose-dependent augmentation of insulin secretion and uptake of nutrients into the adipose tissue.

Materials and methods: *Gipr*$^{-/-}$ and *Gipr*$^{+/+}$ mice were used for lifespan analysis, behavior experiments and gene expression of adipose tissue and muscles. 3T3-L1 differentiated adipocytes were used for Sirt1 and Nampt expression followed by treatment with GIP and α-lipoic acid.

Results: We observed that GIP receptor-knockout (*Gipr*$^{-/-}$) mice fed normal diet showed an extended lifespan, increased exploratory and decreased anxiety-based behaviors, which are characteristic behavioral changes under CR. Moreover, *Gipr*$^{-/-}$ mice showed increased Sirt1 and Nampt expression in the adipose tissue. GIP suppressed α-lipoic acid-induced Sirt1 expression and activity in differentiated adipocytes.

Conclusions: Although maintenance of CR is difficult, food intake and muscle endurance of *Gipr*$^{-/-}$ mice were similar to those of wild-type mice. Inhibition of GIP signaling may be a novel strategy to extend the lifespan of diabetic patients.

1. Introduction

Gastric inhibitory polypeptide (GIP; also known as glucose-dependent insulinotropic polypeptide), secreted by enteroendocrine K cells mainly located in the upper intestine, belongs to incretin family and induces insulin secretion by pancreatic β-cells through GIP receptor (GIPR) in a glucose-dependent manner [1–3].

GIPRs are widely expressed in various organs and tissues such as the brain, bone, adipose tissue, upper intestine, adrenal cortex, and testis [4]. GIP is suggested to exert extra-pancreatic effects. We previously determined the role of GIP in the adipose tissue [5,6]. GIP stimulates lipoprotein lipase activity after decreasing the phosphorylation of 5′-adenosine monophosphate (AMP)-activated protein kinase (AMPK) and promotes fatty acid incorporation into the adipose tissue in the presence of insulin [5,7,8]. GIPR-knockout mice (*Gipr*$^{-/-}$) fed high-fat diet or forced to overeat are resistant to the development of obesity [5]. Therefore, GIP is considered to be an energy storage hormone that promotes obesity after excessive food intake. Interestingly, *Gipr*$^{-/-}$ mice fed normal diet show significantly enhanced spontaneous physical activity, reduced fat mass without reduction in lean body mass or food intake, and improved insulin sensitivity compared with wild-type (*Gipr*$^{+/+}$) mice [9,10]. Moreover, low core body temperature and decreased resting heart rate in *Gipr*$^{-/-}$ mice suggest low activity of the sympathetic nervous system [9] similar to that observed in *Gipr*$^{+/+}$ mice under calorie restriction (CR) or fasting condition.

CR is a dietary regimen that reduces calorie intake without inducing malnutrition or reduction in essential nutrients [11]. CR is associated with longevity and exerts anti-aging effects in various animals [12–15]. Energy limitation through CR or fasting markedly increases the expression and activation of Sirtuins by increasing nicotinamide adenine dinucleotide (NAD^+) levels, decreasing

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https://doi.org/10.1016/j.bbrc.2019.04.036
0006-291X/

NADH levels, or activating AMPK [16,17]. Nicotinamide phosphoribosyltransferase (NAMPT), a rate-limiting enzyme in mammalian NAD^+ biosynthesis is also upregulated in rats under CR or in fasting rats [18].

SIRT1, a mammalian SIR2 orthologue, regulates various metabolic processes in response to changes in nutritional input in multiple tissues [19]. CR modulates SIRT1 level in a tissue-specific manner [20]. Moreover, CR increases the expression and activity of SIRT1, especially in the brain, white adipose tissue, skeletal muscles, and kidneys [21,22]. Sirt1 increases NAMPT expression and activity in adipocytes [19]. Adipocyte-derived extracellular NAMPT (eNAMPT) contributes to SIRT1 activation in the brain [23], which modulates food seeking-associated exploratory behavior in response to CR [24–26].

CR plays a beneficial role in obese diabetic patients [27–30]. However, permanent CR exerts undesirable effects [31], is difficult to maintain, and is enervating, as observed in many patients who withdraw from the regimen. Therefore, a more sustainable treatment than CR is required for treating obese diabetic patients. In the present study, we found that inhibition of GIP signaling increased Sirt1 and Nampt expression in the adipose tissue and hypothalamus. Our results indicate that inhibition of GIP signaling increases longevity with preserved exercise capacity without decreasing food intake.

2. Materials and methods

2.1. Animals

C57BL/6 mice were used to generate *Gipr*$^{-/-}$ and *Gipr*$^{+/+}$ mice, as described previously [32]. *Gipr*$^{-/-}$ and *Gipr*$^{+/+}$ mice were littermates, and the two groups of mice were tested in the same room and at the same period of time. The mice were housed in a controlled environment, with 12-/12-h light/dark cycle, and ad libitum access to standard rodent chow (CE-2; Clea Japan, Tokyo, Japan) and water. Mice were housed one/cage. Food intake (gram per mouse per day) was determined daily over 3 days in mice caged singly. All procedures were approved by the Committee on Animal Experimentation of Akita University. All experiments were performed in accordance with institutional guidelines and were approved by the Committee on Animal Experimentation of Akita University.

2.2. Lifespan analysis

All male mice in aging cohorts were carefully inspected every day. For each mouse, the study endpoint was the time of death, which was determined during daily inspection. Survival data of each cohort were analyzed by plotting Kaplan–Meier curves and by performing a log rank test. Number of lifespan above 80th percentile of two groups was compared in 2×2 table using a one-tailed Boschloo's unconditional exact test. Proportional hazards analysis was performed using mathematical models of mortality, namely, Gompertz, Gompertz-Makeham, logistic, and logistic-Makeham models [33]. R packages Survomatic (Bokov and Gelfond 2012, http://rforge.net/Survomatic/) and Exact (Peter Calhoun 2016, https://cran.r-project.org/package=Exact) were used to fit the mortality models to the observed survival and Boschloo's unconditional exact test, respectively.

2.3. Behavior experiments

Open field test [34]. Mice were placed in the peripheral site of an open field test (OFT) apparatus ($50 \times 50 \times 40$ cm; O'Hara & Co, Tokyo, Japan). Time spent in the center of the apparatus was recorded automatically by using Image OF software (O'Hara & Co). Data were collected for 10 min.

Elevated plus-maze test [35]. Elevated plus-maze (EPM) test apparatus (O'Hara & Co) included two open and two closed arms (25×5 cm). The apparatus was placed at a height of 55 cm. Mice were placed in the center of the apparatus facing one of the closed arms. Time spent in and the number of entries into the open arms were recorded for 10 min.

2.4. Measurement of exercise endurance

11-week-old male *Gipr*$^{+/+}$ and *Gipr*$^{-/-}$ mice were acclimated to a treadmill for 10 min and were made to run at a speed of 20 m/min for 20 min as a test regimen.

2.5. Histological analysis of skeletal muscles

Skeletal muscle samples were frozen in liquid nitrogen-cooled isopentane. Type I and type II skeletal muscle fibers were

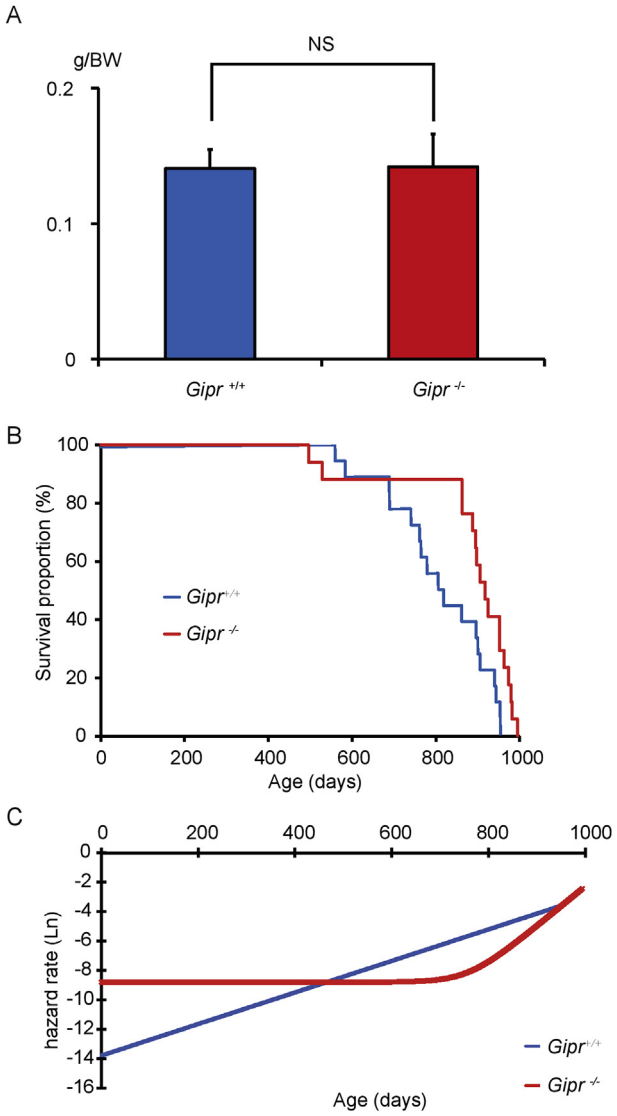


Fig. 1. Caloric intake by and longevity of *Gipr*$^{+/+}$ and *Gipr*$^{-/-}$ mice. (a) Effect of chow diet intake for correcting body weight in 12-week old male *Gipr*$^{+/+}$ (N = 12) and *Gipr*$^{-/-}$ (N = 12) mice. (b) Kaplan–Meier survival curves of *Gipr*$^{-/-}$ (N = 17) and *Gipr*$^{+/+}$(N = 18) male mice. Survival rate was significantly increased in *Gipr*$^{-/-}$ mice compared with that in *Gipr*$^{+/+}$ mice (P = 0.0048, log rank test). (c) Gompertz-Makeham plots of mice in the two groups.

Table 1
Comparison of lifespan above the 80th percentile between *Gipr*$^{+/+}$ and *Gipr*$^{-/-}$ mice.

Lifespan above 80th percentile	*Gipr*$^{+/+}$ mice	*Gipr*$^{-/-}$ mice
$80^{th}<$	0	7
$\leq 80^{th}$	18	10
Total	18	17

P = 0.0021 by one-tailed Boschloo's test.

distinguished by performing myosin ATPase staining at pH 4.3 [36].

2.6. Cell culture

3T3-L1 preadipocytes (ATCC CL-173) were maintained in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, Tokyo, Japan) containing 10% FBS and penicillin–streptomycin (Life Technologies, US) and were incubated at 37 °C in an atmosphere of 5% CO_2. Next, 3T3-L1 preadipocytes were differentiated into mature adipocytes by culturing them in the same medium containing 10 mg/l insulin (Sigma-Aldrich), 1 mol/l dexamethasone (Sigma-Aldrich), and 0.5 mmol/l 3-isobutyl-1-methylxanthine (Sigma-Aldrich) for 2 days. The medium was replaced with fresh DMEM containing 5 mg/l insulin, and the cells were incubated further for 10 days. Mature 3T3-L1 adipocytes were cultured in DMEM with or without 1 mmol/l α-lipoic acid (LA; Sigma-Aldrich) for 24 h, followed by treatment with 100 nmol/l GIP for the indicated time. SIRT1 activity in 3T3-L1 adipocytes was measured using SIRT1 Activity Assay Kit (ab156065; Abcam, GB), according to the manufacturer's instructions.

2.7. RNA isolation and analysis

Total RNA from the brain, white adipose tissue (WAT), and mature 3T3-L1 adipocytes was isolated using RNeasy lipid tissue mini-kit (Qiagen, CA) and that from the skeletal muscles and liver was isolated using RNeasy mini-kit (Qiagen). cDNA was synthesized using PrimeScript™ First-Strand cDNA Synthesis Kit (Takara Bio Inc., Japan). Quantitative RT-PCR was performed using SYBR Green I Kit (Roche, CH) or Assay-on-Demand TaqMan primer/probe sets (Roche). Primer information is provided in Supplemental Table 1. Relative expression levels were determined based on Ct values and were normalized to the expression of the 18S ribosomal RNA gene.

2.8. Statistics

All values unless otherwise noted are presented as mean ± standard deviation (SD). Comparison of two groups was performed using unpaired Student's *t*-test (two-tailed) or

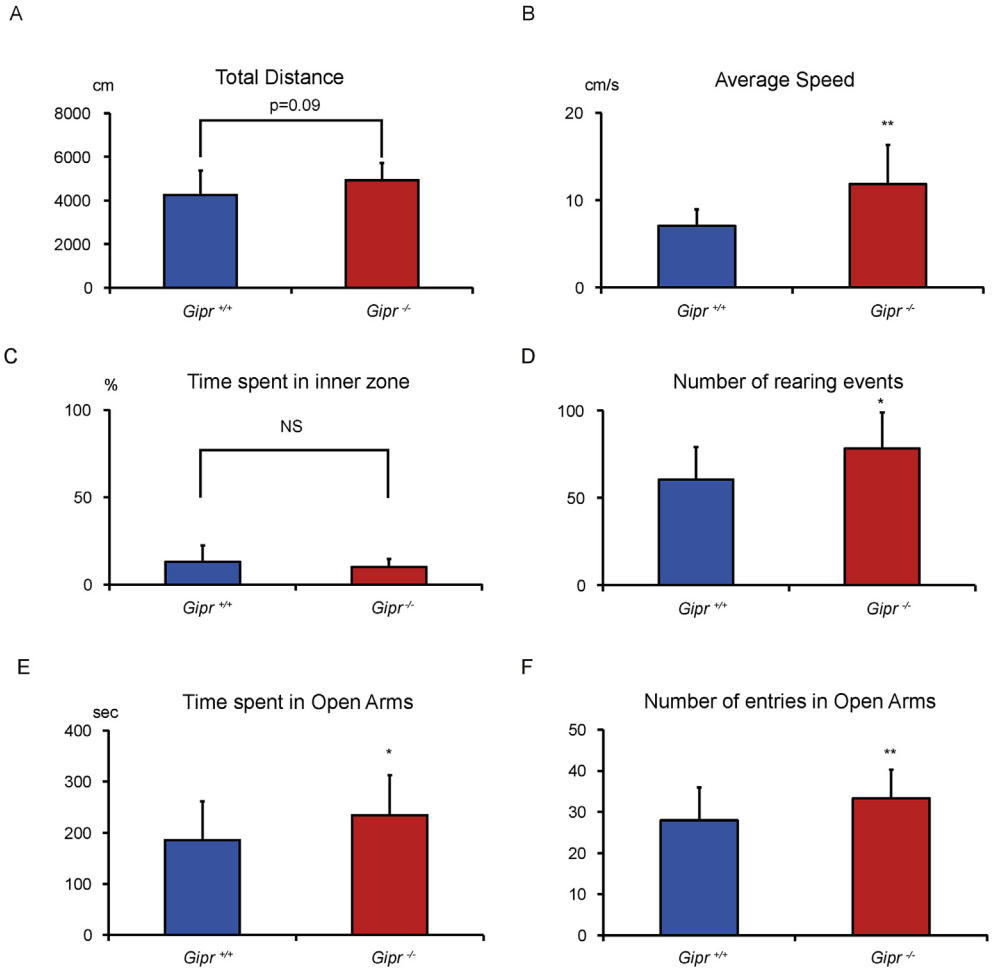


Fig. 2. Spontaneous behavior of *Gipr*$^{+/+}$ and *Gipr*$^{-/-}$ mice. Measurement of spontaneous behavior by performing the OFT. Total Distance (a), average speed (b), time spent in inner zone (c), and number of rearing events (d) were measured for 10 min in 8–10-week-old male *Gipr*$^{+/+}$ (N = 14) and *Gipr*$^{-/-}$ (N = 12) mice. Data are expressed as mean ± SD; *P < 0.05 and **P < 0.01; NS, not significant. Measurement of spontaneous behavior by performing the EPM test. Time spent in open arms (e) and number of entries in open arms (f) were measured for 10 min in 6-7-week-old *Gipr*$^{+/+}$ (N = 28) and *Gipr*$^{-/-}$ (N = 31) male mice. In this test, anxiety-based behavior was defined by the less amount of time spent in and few number of entries into the open arms. Data are expressed as mean ± SD; *P < 0.05 and **P < 0.01.

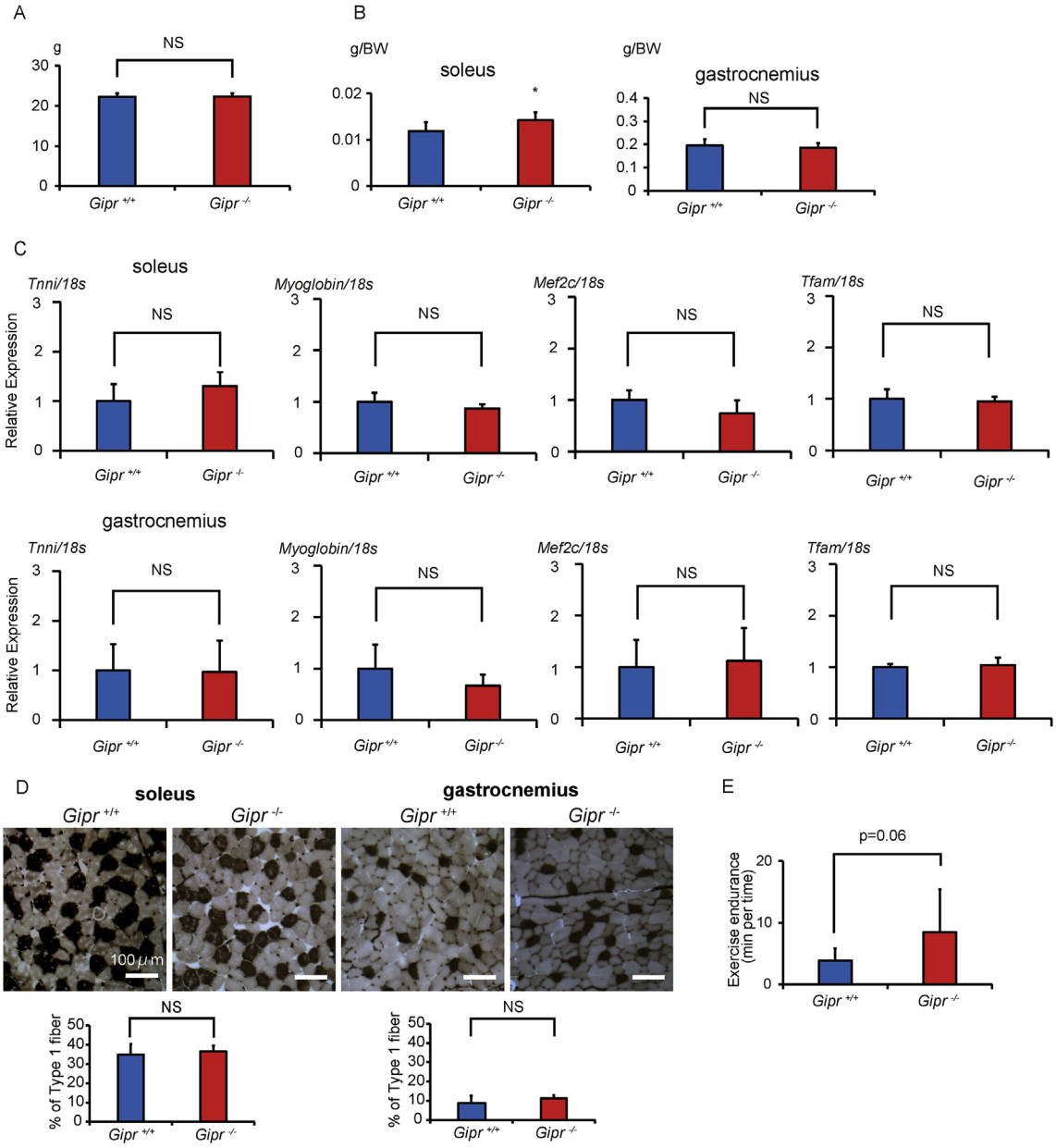


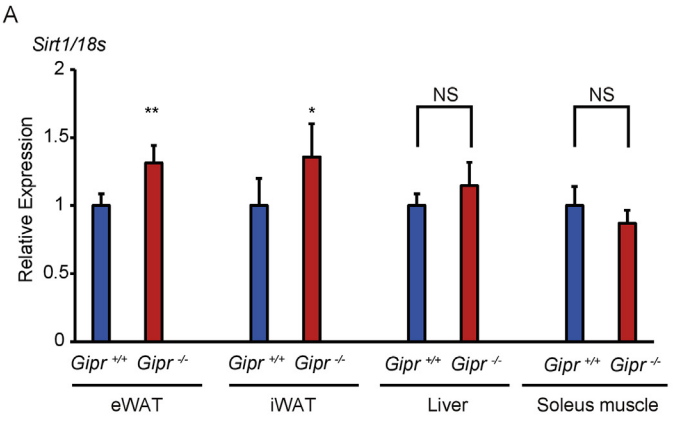
Fig. 3. Quality and quantity of skeletal muscles in *Gipr*$^{+/+}$ and *Gipr*$^{-/-}$ mice. (a) Body weight of 11-week-old male *Gipr*$^{+/+}$ and *Gipr*$^{-/-}$ mice. (b) Mass of the soleus (slow-twitch fiber) and gastrocnemius muscles (fast-twitch fiber) after correcting for body weight. (c) Expression of genes encoding skeletal muscle markers in 11-week-old male *Gipr*$^{+/+}$ and *Gipr*$^{-/-}$ mice. The mRNA levels of genes encoding myoglobin, TNNI1, MEF2C, and TFAM were determined in the soleus and gastrocnemius muscles. (d) ATPase staining (at pH 4.3 for type I fibers) of the soleus muscle (original magnification, × 50; scale bar, 100 μm), and quantification of type I fibers based on fiber-type analyses. (e) Treadmill running test for assessing exercise endurance. Exercise endurance was assessed by dividing 20 min by the number of times a mouse was unable to avoid electrical shocks. All values are presented as mean ± SD (N = 5–7); *P < 0.05 and **P < 0.01 compared with *Gipr*$^{+/+}$ mice; NS, not significant.

Mann–Whitney *U* test for nonparametric data. Comparison of more than two groups was performed using two-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test or one-way ANOVA followed by Tukey-Kramer's post-test to evaluate statistical significance. P < 0.05 was considered statistically significant. All statistical analyses except survival analysis were performed using a Bell Curve for Excel and SPSS 22.0 (IBM Japan Ltd, Tokyo, Japan).

3. Results

3.1. *Gipr*$^{-/-}$ mice showed significantly prolonged survival

CR extends the lifespan of various animals [12–15]. Intake of regular chow was similar in *Gipr*$^{-/-}$ and *Gipr*$^{+/+}$ mice (Fig. 1A). However, *Gipr*$^{-/-}$ mice showed significant lifespan extension compared with *Gipr*$^{+/+}$ mice (P = 0.0048, log rank test) (Fig. 1B). *Gipr*$^{-/-}$ mice showed extension in median lifespan (*Gipr*$^{+/+}$, 806 days versus *Gipr*$^{-/-}$, 917 days; p = 0.0191 by *U* test) and in the maximum lifespan by 44 days (*Gipr*$^{+/+}$, 951 days versus *Gipr*$^{-/-}$, 995 days) (Fig. 1B). To test the hypothesis that maximum lifespan is longer in *Gipr*$^{-/-}$ mice than in *Gipr*$^{+/+}$ mice, number of lifespan above the 80th percentile was compared between groups (Table 1) [37], indicating P = 0.0021 by Boschloo's test. Age-specific hazard rates in *Gipr*$^{-/-}$ and *Gipr*$^{+/+}$ mice were also investigated in parametric model using maximum-likelihood method. The Gompertz-Makeham model showed a better fit than the other models.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Sirt1/18s
Relative Expression
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NS
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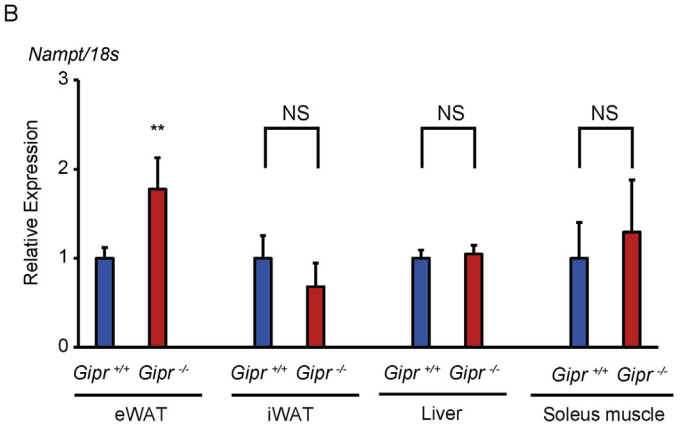
Liver
Soleus muscle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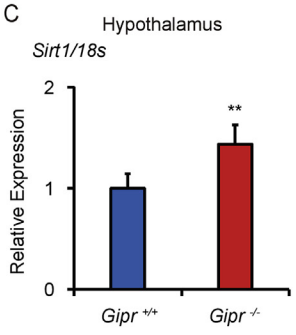
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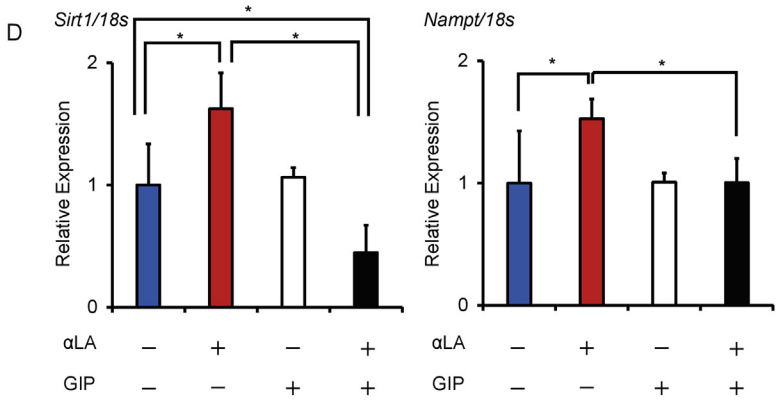
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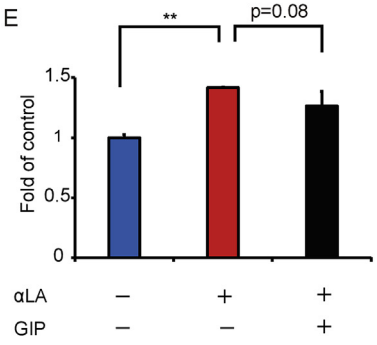
E
Fold of control
1.5
1
0.5
0
**
p=0.08
αLA – + +
GIP – – +

Predicted hazard rates characterized using three parameters, namely, λ, γ, and c (referred to as initial mortality parameter, rate parameter, and constant, respectively), are plotted in Fig. 1C. The λ and γ parameters and the combination of the three parameters were significantly different between *Gipr*$^{+/+}$ and *Gipr*$^{-/-}$ mice (λ: 1.02×10^{-6} versus 1.78×10^{-13}, $P = 0.0013$; γ: 1.07×10^{-2} versus 2.71×10^{-2}, $P = 0.0003$ and the combination of the three parameters: $P = 0.0021$, respectively).

3.2. *Gipr*$^{-/-}$ mice showed novelty seeking tendency

To confirm whether *Gipr*$^{-/-}$ and *Gipr*$^{+/+}$ mice showed different changes in the behavior pattern, we evaluated their exploratory and anxiety-based behaviors. The OFT was performed to evaluate the degree of novelty seeking. The number of rearings and average speed significantly increased in *Gipr*$^{-/-}$ mice (Fig. 2A–D). The EPM test was performed to assess the exploratory and anxiety-based behaviors of *Gipr*$^{-/-}$ and *Gipr*$^{+/+}$ mice. Time spent exploring the open arms and the number of entries into these arms were significantly higher for *Gipr*$^{-/-}$ mice than for *Gipr*$^{+/+}$ mice (Fig. 2E and F).

These results indicate that *Gipr*$^{-/-}$ mice showed significantly increased exploratory behavior and decreased anxiety-based behavior.

3.3. *Gipr*$^{-/-}$ mice maintained exercise endurance

Previous studies suggest that low dietary protein intake in older adults induces the loss of skeletal muscles [31,38]. We measured skeletal muscle mass and composition in *Gipr*$^{-/-}$ mice. As shown in the results of the previous study [5], no difference was observed in the body weights of *Gipr*$^{+/+}$ and *Gipr*$^{-/-}$ mice (Fig. 3A). The mass of the gastrocnemius muscle was comparable between *Gipr*$^{-/-}$ and *Gipr*$^{+/+}$ mice; however, the mass of the soleus muscle was significantly higher in *Gipr*$^{-/-}$ mice than in *Gipr*$^{+/+}$ mice (Fig. 3B). Expression levels of genes encoding type I fiber differentiation factors such as myocyte enhancer factor 2C (MEF2C) and mitochondrial transcription factor A (TFAM) and type I fiber markers such as troponin I (slow) were similar between *Gipr*$^{-/-}$ and *Gipr*$^{+/+}$ mice (Fig. 3C). Histological analysis showed that the composition of type I fibers was comparable between *Gipr*$^{-/-}$ and *Gipr*$^{+/+}$ mice (Fig. 3D).

To determine the effect of GIPR ablation on skeletal muscle function in live animals, we determined the muscle endurance in *Gipr*$^{+/+}$ and *Gipr*$^{-/-}$ mice by making them perform an involuntary physical exercise, i.e., running on a treadmill. Muscle endurance was slightly but not significantly higher in *Gipr*$^{-/-}$ mice than in *Gipr*$^{+/+}$ mice ($P = 0.06$; Fig. 3E).

These results indicate that disruption of GIP signaling does not exert any harmful effects on the skeletal muscles.

3.4. GIP regulated the expression of genes encoding SIRT1 and NAMPT in the adipose tissue

Because CR upregulates SIRT1 and NAMPT expression [16–18], we examined the mRNA levels of genes encoding SIRT1 and NAMPT in *Gipr*$^{-/-}$ mice. We found that the mRNA levels of genes encoding SIRT1 and NAMPT were significantly increased in the epididymal fat of *Gipr*$^{-/-}$ mice ($P < 0.01$ and $P < 0.01$, respectively; Fig. 4A and B). Expression of the gene encoding SIRT1 was also upregulated in the inguinal fat of *Gipr*$^{-/-}$ mice ($P < 0.05$; Fig. 4A). However, no obvious difference was observed in the expression of the gene encoding NAMPT (Fig. 4B). Moreover, mRNA levels of the genes encoding SIRT1 and NAMPT in the skeletal muscles and liver were comparable between *Gipr*$^{-/-}$ and *Gipr*$^{+/+}$ mice (Fig. 4A and B). Interestingly, the mRNA level of the gene encoding SIRT1 was significantly elevated in the hypothalamus of *Gipr*$^{-/-}$ mice ($P < 0.01$; Fig. 4C).

To determine whether GIP signaling directly affected SIRT1 and NAMPT expression, 3T3-L1 differentiated adipocytes were treated with GIP. The mRNA expression of the gene encoding SIRT1 in 3T3-L1 differentiated adipocytes increased after pretreatment with α-lipoic acid (LA), which increases intracellular NAD^+ levels and SIRT1 production and activity (Fig. 4D and E). GIP alone did not affect the expression of the gene encoding SIRT1 and the activity of SIRT1. However, GIP treatment after LA pretreatment decreased the expression of the gene encoding SIRT1 ($P < 0.05$) and the deacetylating activity of SIRT1 ($P = 0.08$) (Fig. 4D and E). Expression of the gene encoding NAMPT, which is modulated by SIRT1 [39], also significantly decreased after LA pretreatment and GIP treatment (Fig. 4D).

These results indicate that GIP signaling suppresses LA-induced overexpression of genes encoding SIRT1 and NAMPT in adipocytes.

4. Discussion

Similarities and differences between mice under CR and *Gipr*$^{-/-}$ mice are summarized in Table 2 [9,40–43]. *Gipr*$^{-/-}$ mice showed increased lifespan, locomotor activity, and novelty seeking tendency.

CR is one of the established method to extend lifespan. Kaplan–Meier analyses of rodents under CR show right-shifted survival curves and increased median lifespan. Further examination by using a mathematical model indicates that reduction of age-associated increase in mortality rate is crucial for extending the lifespan of rodents under CR [33]. Age-specific hazard rates of *Gipr*$^{-/-}$ and *Gipr*$^{+/+}$ mice were determined using the maximum-likelihood method with the Gompertz, Gompertz-Makeham, logistic, and logistic-Makeham mortality models. The Gompertz-Makeham model showed a better fit than the other models. *Gipr*$^{-/-}$ mice showed significantly lower age-specific hazard rates than *Gipr*$^{+/+}$ mice, which was consistent with the results obtained in rodents under CR.

Decreased age-specific hazard rates have also been observed in several animal models such as adipocyte-specific insulin receptor-knockout mice [44] and brain-specific SIRT1-overexpressing (BRASTO) mice [26]. BRASTO mice also show increased spontaneous physical activity [26]. SIRT1 expression was elevated in the hypothalamus of *Gipr*$^{-/-}$ mice. SIRT1 expression may be increased in *Gipr*$^{-/-}$ mice because of two reasons. First, GIPR, which is widely expressed in the central nervous system [45], directly or indirectly inactivates orexin nervous system. Orexin signaling mediates the antidepressant effects of CR [46]. Moreover, orexin neuron-ablated mice do not respond to fasting with increased wakefulness and activity [47], indicating that increased locomotor activity and exploratory behavior are associated with increased SIRT1 activity through orexin signaling in the hypothalamus. However, the

Fig. 4. GIP-induced regulation of SIRT1 expression and activity. The mRNA levels of genes encoding SIRT1 and NAMPT in *Gipr*$^{+/+}$ and *Gipr*$^{-/-}$ mice. The mRNA levels of the genes encoding SIRT1 (a) and NAMPT (b) in the epididymal fat (eWAT), inguinal fat (iWAT), liver, and soleus muscles and the mRNA level of the gene encoding SIRT1 in the hypothalamus (c) of *Gipr*$^{+/+}$ and *Gipr*$^{-/-}$ mice; N = 5–6 per group; $P < 0.05$ and $^{**}P < 0.01$ compared with *Gipr*$^{+/+}$ mice; NS, not significant. The mRNA levels of the genes encoding SIRT1 and NAMPT (d) and the deacetylating activity of SIRT1 (e) in 3T3-L1 adipocytes treated with GIP for 3 h. All values are presented as mean ± SD; N = 3–4 per group; $^{*}P < 0.05$ and $^{**}P < 0.01$.

Table 2
Similarities and differences between mice under CR and *Gipr*$^{-/-}$mice.

	MMice under CR	*GGipr*$^{-/-}$ mice
Lifespan	↑	↑
Exploratory behavior	↑	↑
Body temperature	↓	↓
Heart rate	↓	↓
Adipose tissue SIRT1 and NAMPT	↑	↑
Insulin sensitivity	↑	↑
Skeletal muscle mass	→ ~ ↓	→ ~ ↑
Food intake	↓	→

relative importance of GIP in the central versus peripheral-derived GIP is unclear. Therefore, further studies are needed to determine its mechanism of action. Second, adipocyte-derived eNAMPT increases the activity of orexin receptor type 2, a downstream target of SIRT1 activation in the hypothalamus, through blood circulation [23,48]. Adipocyte-derived eNAMPT functions as an adipokine [49] and the dimeric form of eNAMPT may affect hypothalamic neuron activity [23]. Because SIRT1 and NAMPT expression levels are elevated in the adipose tissue of *Gipr*$^{-/-}$ mice and because GIP suppresses SIRT1 expression and activity in 3T3-L1 adipocytes, SIRT1 expression in the hypothalamus of *Gipr*$^{-/-}$ mice may be elevated because of the desuppression of eNAMPT in the adipocytes of *Gipr*$^{-/-}$ mice.

Food intake is remarkably different between mice under CR and *Gipr*$^{-/-}$ mice. Our data indicate that food intake is restricted under CR but remains unchanged after *Gipr* knockdown. In addition, CR might reduce muscle strength and aerobic capacity ($\dot{V}O_2$ max) [31]. Although we did not measure muscle strength and $\dot{V}O_2$ max in the present study, we assessed the muscle mass of the gastrocnemius (fast-twitch fiber) and soleus muscles (slow-twitch fiber) and determined exercise endurance by performing the treadmill test in *Gipr*$^{-/-}$ mice. We found that the muscle mass of the gastrocnemius and soleus muscles and exercise endurance were preserved in *Gipr*$^{-/-}$ mice compared with those in *Gipr*$^{+/+}$ mice. The reason why the muscle mass of soleus muscle in *Gipr*$^{-/-}$ mice was significantly greater than that in *Gipr*$^{+/+}$ mice is unclear, but it might be due to a large amount of locomotor activity [9]. Thus, suppression or blockade of GIP signaling might mimic the effects of CR without reducing food intake, skeletal muscle mass, and exercise endurance.

Dipeptidyl peptidase-4 (DPP-4) inhibitors are widely used as therapeutic agents for diabetes to improve glycemic control by promoting insulin secretion [50]. DPP-4 inhibitors decrease blood glucose levels and increase plasma insulin levels in GLP-1 receptor-knockout mice. However, these effects of DPP-4 inhibitors are suppressed in GIP receptor- and GLP-1 receptor-knockout mice [51], indicating that augmented GIP signaling plays a crucial role in inducing the anti-hyperglycemic effects of DPP-4 inhibitors. The literature is controversial about agonism or antagonism of GIP signaling could be beneficial for the obesity and diabetes treatment. Another work suggests that use of agonist could be beneficial for these pathologies [52]. On the other hand, we and other groups have shown that suppression of GIP signaling by genetic engineering and use of a GIPR antagonist or anti-GIP antibody may be an option for treating obesity and diabetes [5,53,54]. Previously, we showed that the use of α-glucosidase inhibitors suppresses GIP secretion, leading to weight loss in diabetic patients [55,56]. The α-glucosidase inhibitor had been shown to extend lifespan in mice [57]. Because CR in obese diabetic patients exerts beneficial but undesirable effects and is difficult to maintain, prevention of GIP signaling by administering α-glucosidase inhibitors may be an alternative option to reduce body weight and extend lifespan of these patients.

Conflicts of interest

No potential conflicts of interest relevant to this article were reported.

Funding

This study was supported in part by Grants-in-Aid for Scientific Research (B) (22390183 To Y.Y.) from the Ministry of Education, Culture, Sports, Science and Technology and Science and technology research promotion program for agriculture, forestry, fisheries and food industry (grant nos. 28029C) from Ministry of Agriculture, Forestry and Fisheries, Japan. This study was also supported by practical research project for life-style related diseases including cardiovascular diseases and diabetes mellitus from Japan Agency for Medical Research and Development and grants-in-aid for scientific research from Japan Society for the Promotion of Science.

Acknowledgements

We thank Ms. Kaoru Sakamoto, Ms. Kayoko Kagaya and Ms. Hiromi Fujishima for providing technical support. We would like to thank Editage (www.editage.jp) for English language editing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2019.04.036.

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