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Genetic inactivation of GIP signaling reverses aging-associated insulin resistance through body composition changes

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Abstract

Aging is associated with increased fat mass and decreased lean mass, which is strongly associated with the development of insulin resistance. Gastric inhibitory polypeptide (GIP) is known to promote efficient storage of ingested nutrients into adipose tissue; we examined aging-associated changes in body composition using 10-week-old and 50-week-old wild-type (WT) and GIP receptor knockout ($Gipr^{-/-}$) mice on a normal diet, which show no difference in body weight. We found that $Gipr^{-/-}$ mice showed significantly reduced fat mass without reduction of lean mass or food intake, while WT mice showed increased fat mass and decreased lean mass associated with aging. Moreover, aged $Gipr^{-/-}$ mice showed improved insulin sensitivity, which is associated with amelioration in glucose tolerance, higher plasma adiponectin levels, and increased spontaneous physical activity. We therefore conclude that genetic inactivation of GIP signaling can prevent the development of aging-associated insulin resistance through body composition changes.

Keywords: GIP; Body composition; Fat mass; Lean mass; Aging; Insulin resistance; Physical activity; GIP antagonism

Aging is associated with an increase in fat mass, thought to result from a sedentary lifestyle and *ad libitum* food intake over a prolonged period. Age-related accumulation of visceral fat is strongly associated with the development of insulin resistance [1–3]. On the other hand, reduction in skeletal muscle mass or sarcopenia also is a common feature of aging [4,5], increasing the risk for the development of insulin resistance [6]. To prevent the development of insulin resistance, various diet and exercise intervention trials have been conducted to favorably modify body composition by reducing fat mass while maintaining lean mass.

Gastric inhibitory polypeptide (GIP) is secreted from duodenal endocrine K cells in response to meal ingestion as an incretin, potentiating glucose-induced insulin secretion. Functional GIP receptors are expressed in adipose tissue [7] as well as in pancreatic β-cells, and GIP has been known to stimulate lipoprotein lipase activity and promote fatty acid incorporation into adipose tissue in the presence of insulin in cultured adipocytes [8–10]. Since GIP secretion is most strongly stimulated by fat ingestion [11,12], a high-fat diet is considered to be an ideal metabolic stress to induce hypersecretion of GIP for observation of

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subsequent GIP action on adipocytes. The importance of GIP signaling on fat accumulation *in vivo* was first reported by Miyawaki et al. in mice with a targeted disruption of the GIP receptor gene (Gipr$^{-/-}$ mice), which exhibited reduced adiposity on a high-fat diet [9]. Since there was no difference in body weight between wild-type (WT) and Gipr$^{-/-}$ mice on a normal diet during a 50-week observation period [9], no attempt has been made to further investigate long-term GIP action in adipose tissue under normal nutritional conditions.

In the present study, we examined aging-associated changes in body composition using 10-week-old and 50-week-old WT and Gipr$^{-/-}$ mice on a normal diet. As in the previous study [9], the body weight of WT and Gipr$^{-/-}$ mice was almost identical, but computed tomography (CT) based-body composition analysis revealed that 50-week-old Gipr$^{-/-}$ mice had dramatically reduced fat mass and sustained lean mass compared with 50-week-old WT mice, which showed an age-related increase in fat mass and a decrease in lean mass.

Materials and methods

Animals. Generation of Gipr$^{-/-}$ mice was previously described [13]. Ten-week-old and 50-week-old male Gipr$^{-/-}$ mice and littermate WT controls on a C57BL/6 background were used. The animals had *ad libitum* access to standard rodent chow and water. Food intake (gram per mouse per day) was determined daily over 5 days in mice caged singly. All procedures were approved by the Animal Care Committee of Kyoto University Graduate School of Medicine.

CT-based body composition analysis. The mice were anesthetized with intraperitoneal injection of pentobarbital sodium (Dainippon Pharmaceutical, Japan) and their whole bodies were scanned along the body axis using the LaTheta (LCT-100M) experimental animal CT system (Aloka, Japan). Contiguous 1-mm slice images of the body including trunk and lower extremities were used for quantitative assessment using LaTheta software (version 1.00). Weights of total fat mass, which consists of visceral fat mass plus subcutaneous fat mass, and lean mass were determined and normalized by body weight.

Plasma hormone measurements. Plasma insulin, leptin, and adiponectin levels were determined by ELISA kits for mouse insulin (Shibayagi, Japan), mouse leptin (Morinaga, Japan), and mouse/rat adiponectin (Otsuka Pharmaceutical, Japan), respectively.

Insulin and glucose tolerance tests. For insulin tolerance test (ITT), 0.4 U/kg human insulin (Novonordisk, Denmark) was injected intraperitoneally after 5-h fasting. Oral glucose tolerance test (OGTT) was carried out following an overnight fast (16 h) and 2.0 g/kg glucose was loaded. Blood samples were taken at indicated times and blood glucose levels were measured by the enzyme-electrode method. HbA_{1c} was measured by immunoassay (DCA 2000 system, Bayer Diagnostics).

Insulin secretion from isolated islets. Isolation of pancreatic islets and batch incubation experiments were performed as described previously [14]. Briefly, 10 islets were collected in each tube and pre-incubated at 37 °C for 30 min in the medium containing 2.8 mM glucose, and incubated for another 30 min in the medium containing the indicated concentrations of glucose with 10^{-7} M human GIP or GLP-1 (Peptide Institute, Inc., Japan). Insulin secretion was measured by RIA using mouse insulin as a standard.

Telemetry recordings. Twelve- to 18-week-old WT and Gipr$^{-/-}$ mice weighing 27–30 g were used. The mice were anesthetized with pentobarbital sodium, and a small telemetric transmitter (TA10ETA-F20, Data Sciences Inc., USA) was implanted into the abdominal cavity. Seven to 14 days of recovery from the surgery was allowed before initiation of data collection. The mice were left undisturbed under a light/dark cycle of 14 h/10 h (lights on at 07:00 h and lights off at 21:00 h), and telemetry recordings for motor activity, body temperature (BT), and heart rate (HR) were performed every 2 min and averaged in 1-h bins using Dataquest A.R.T. software (version 2.1) (Data Sciences Inc.). The average for each bin from the same time point during a consecutive 5-day observation period was used for calculation.

Statistical analysis. Results are expressed as means ± SE. Statistical significance was assessed by ANOVA and unpaired Student's *t*-test, where appropriate. A P value of <0.05 was considered to be statistically significant.

Results

Aged Gipr$^{-/-}$ mice had reduced fat mass and sustained lean mass independent of changes in body weight or food intake

Body weight of WT and Gipr$^{-/-}$ mice was almost identical throughout the 50-week observation period (Fig. 1A). Body lengths measured at 10 and 50 weeks of age were also almost the same (data not shown). There was no difference in food intake between WT and Gipr$^{-/-}$ mice (Fig. 1B). CT-based analyses of body composition were performed as shown in Fig. 2A. There was no apparent difference in representative CT images showing abdominal fat (Fig. 2B, a) and thigh muscle (Fig. 2B, b) of 10-week-old WT and Gipr$^{-/-}$ mice. However, 50-week-old Gipr$^{-/-}$ mice had markedly less fat mass and a greater proportion of lean mass compared with 50-week-old WT mice (Fig. 2C). Total, subcutaneous, and visceral fat mass was similar in 10-week-old WT and Gipr$^{-/-}$ mice, but there

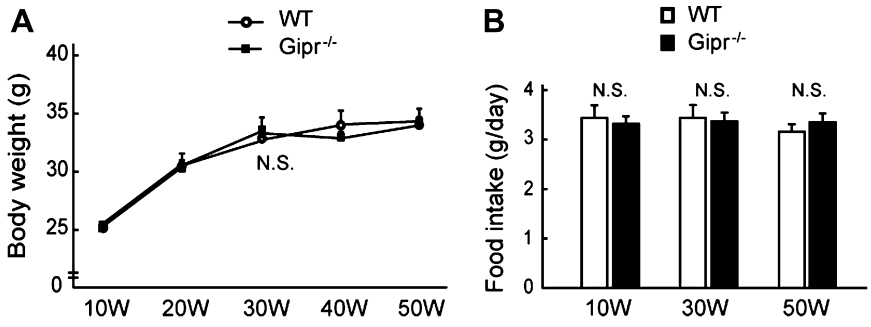

Fig. 1. Body weight and food intake. (A) Body weight of WT and Gipr$^{-/-}$ mice during the 50-week observation period. (B) Food intake (g/day) for each mouse was measured at 10, 30, and 50 weeks. $n = 6$–15 mice/group.

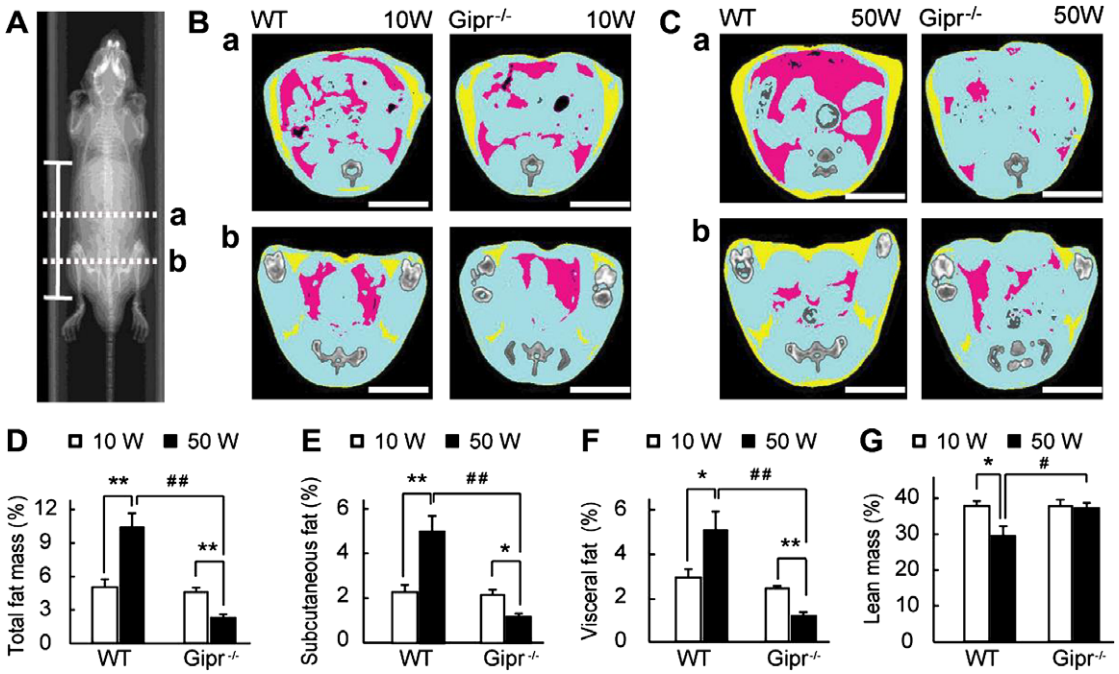

Fig. 2. CT-based body composition analyses. (A) The solid white bar indicates the observation area. Representative CT images showing abdominal fat (a) and thigh muscle (b) of 10-week-old (B) and 50-week-old (C) WT and Gipr$^{-/-}$ mice. The pink, yellow, and light blue areas represent visceral fat, subcutaneous fat, and lean mass, respectively. (D) Total fat mass, (E) subcutaneous fat mass, (F) visceral fat mass, and (G) lean mass, normalized to body weight, in 10-week-old and 50-week-old WT and Gipr$^{-/-}$ mice. $n = 6$–8 mice/group. $^{*}P < 0.05$; $^{**}P < 0.01$, 10-week-old vs 50-week-old mice, $^{\#}P < 0.05$; $^{\#\#}P < 0.01$, WT vs Gipr$^{-/-}$ mice. Scale bars, 1 cm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

was a considerable difference in fat mass between 50-week-old WT and Gipr$^{-/-}$ mice (Fig. 2D–F). On the other hand, the weight of lean body mass was significantly increased in 50-week-old Gipr$^{-/-}$ mice (weights of lean body mass: 9.6 ± 0.5, 9.7 ± 0.6, 10.1 ± 0.7, and 12.4 ± 0.5 g for 10-week-old WT, 10-week-old Gipr$^{-/-}$, 50-week-old WT, and 50-week-old Gipr$^{-/-}$ mice, respectively, $P < 0.05$, 50-week-old WT vs Gipr$^{-/-}$ mice, $P < 0.01$, 10-week-old vs 50-week-old Gipr$^{-/-}$ mice). When normalized by body weight, lean mass was significantly decreased in 50-week-old WT mice, while 50-week-old Gipr$^{-/-}$ mice maintained the same percentage of lean mass as the young mice (Fig. 2G). There was no difference in organ weight between 50-week-old WT and Gipr$^{-/-}$ mice (liver weight: WT 1.64 ± 0.12 g vs Gipr$^{-/-}$ 1.63 ± 0.10 g, intestinal weight: WT 3.69 ± 0.21 g vs Gipr$^{-/-}$ 3.53 ± 0.40 g) as well as in 10-week-old WT and Gipr$^{-/-}$ mice (data not shown).

Aged Gipr$^{-/-}$ mice showed improved insulin sensitivity and amelioration in glucose tolerance

Insulin sensitivity was evaluated by ITT, and the glucose-lowering effect of insulin was decreased in 50-week-old WT mice compared with 10-week-old WT mice, indicating that WT mice had developed age-related insulin resistance (Fig. 3A). To the contrary, 50-week-old Gipr$^{-/-}$ mice were more insulin sensitive than 10-week-old Gipr$^{-/-}$ mice (Fig. 3B). There was no difference in glucose tolerance between 10-week-old and 50-week-old WT mice (Fig. 3C), but in 50-week-old WT mice, a compensatory increase in insulin secretion was required to achieve the same blood glucose levels as those in 10-week-old WT mice (Fig. 3E). In 50-week-old Gipr$^{-/-}$ mice, fasting and 15-min glucose levels were significantly decreased compared with 10-week-old Gipr$^{-/-}$ mice (76.4 ± 4.6 vs 56.0 ± 9.1 mg/dl at 0 min, $P < 0.01$, and 386.8 ± 20.6 vs 349.7 ± 46.0 mg/dl at 15 min, $P < 0.05$, for 10-week-old vs 50-week-old Gipr$^{-/-}$ mice, respectively), and the glycemic excursion between 30 min and 120 min was lower than that of 10-week-old Gipr$^{-/-}$ mice (Fig. 3D), although plasma insulin levels were not increased (Fig. 3F). Although Gipr$^{-/-}$ mice exhibited elevated post-challenge blood glucose levels, overall glycemic control as shown by HbA_{1c} was not worse in Gipr$^{-/-}$ mice compared with that in WT mice (3.10 ± 0.16, 2.84 ± 0.12, 2.80 ± 0.11, and $2.65 \pm 0.1\%$ for 10-week-old WT, 10-week-old Gipr$^{-/-}$, 50-week-old WT, and 50-week-old Gipr$^{-/-}$ mice, respectively). We also determined insulin secretion from isolated islets and found that the insulin secretory response to glucose and GLP-1 stimulation was intact in 10-week-old and 50-week-old Gipr$^{-/-}$ mice compared with their WT controls (Fig. S1(A) and S1(B)).

Aged Gipr$^{-/-}$ mice showed favorable changes in plasma adipocytokine levels and spontaneous hyperactivity

Consistent with fat mass, plasma leptin levels were significantly lower in 50-week-old Gipr$^{-/-}$ mice than in

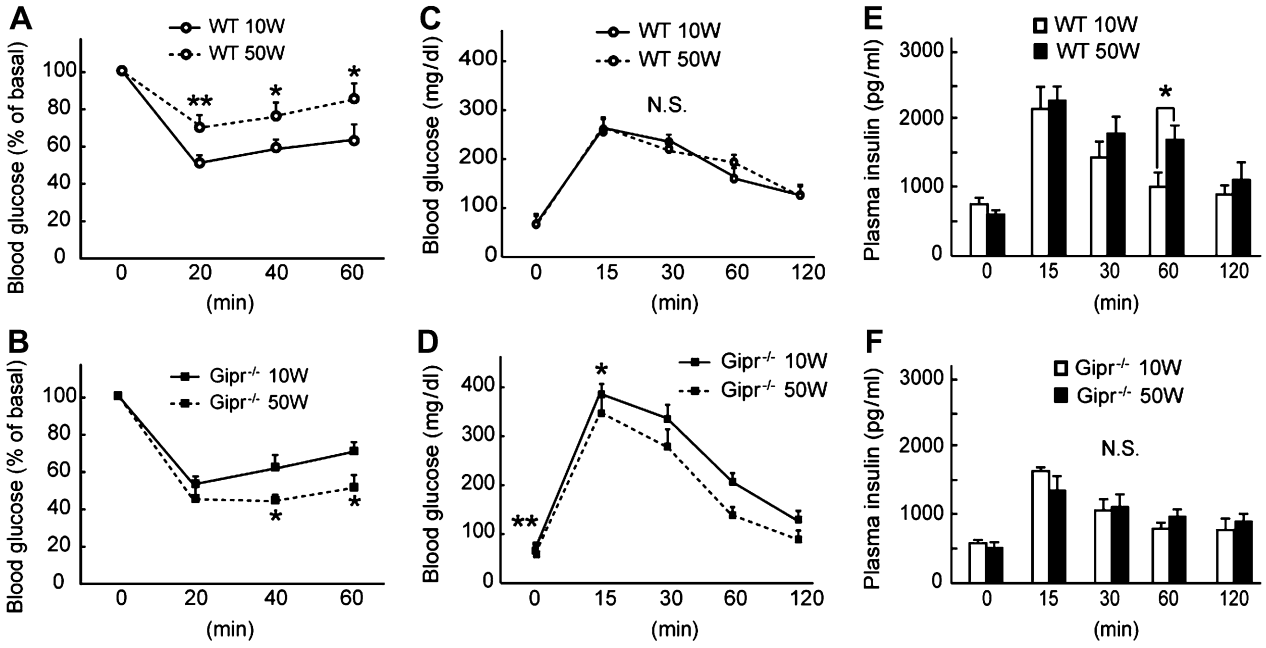

Fig. 3. Insulin and glucose tolerance tests. Results of insulin tolerance tests in 10-week-old and 50-week-old WT (A) and Gipr$^{-/-}$ (B) mice. $n = 6$–10 mice/group. Blood glucose levels during oral glucose tolerance test (OGTT) in 10-week-old and 50-week-old WT mice (C) and 10-week-old and 50-week-old Gipr$^{-/-}$ mice (D). Plasma insulin levels during OGTT (B) in 10-week-old and 50-week-old WT mice (E) and 10-week-old and 50-week-old Gipr$^{-/-}$ mice (F). $n = 5$–8 mice/group. $^{*}P < 0.05$; $^{**}P < 0.01$, 10-week-old vs 50-week-old mice.

50-week-old WT mice (Fig. 4A). On the other hand, plasma adiponectin levels were significantly higher in 50-week-old Gipr$^{-/-}$ mice than in 50-week-old WT mice (Fig. 4B). To investigate the underlying mechanism of increased lean mass, we used implanted telemetry chips to measure the 24-h profile of physical activity. WT and Gipr$^{-/-}$ mice both exhibited a robust rhythm of physical activity with intense activity during the dark phase and rest during the light phase (Fig. 4C). The average activity counts during a consecutive 5-day observation period showed that spontaneous activity was significantly increased in Gipr$^{-/-}$ mice both in light and dark phases

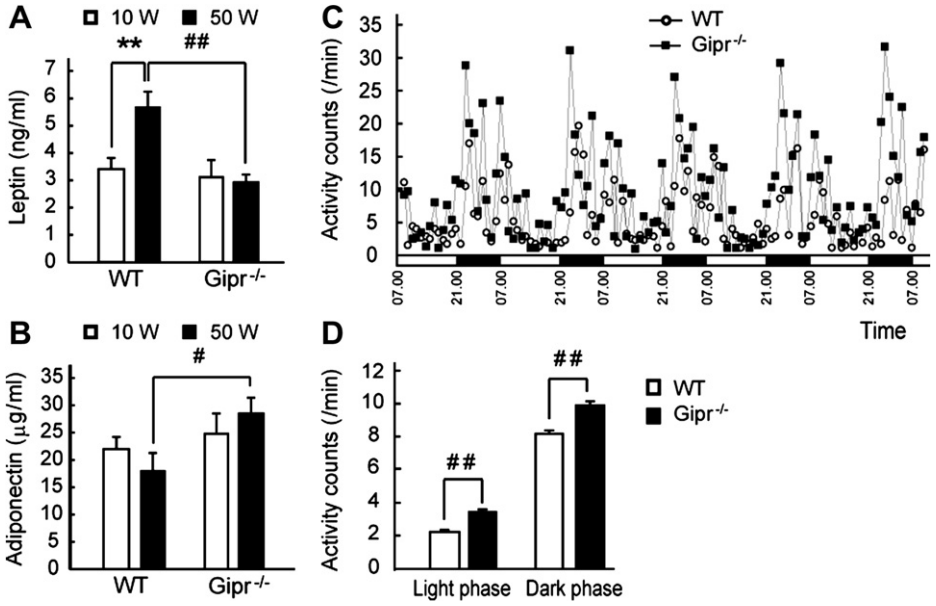

Fig. 4. Plasma adipocytokine levels and physical activity. (A) Plasma leptin and (B) adiponectin levels in 10-week-old and 50-week-old WT and Gipr$^{-/-}$ mice. $n = 6$–10 mice/group. (C) Representative circadian patterns of physical activity in a WT and Gipr$^{-/-}$ mouse under a 14 h/10 h light/dark cycle during a consecutive 5-day observation period. The white and black bar below the figure represents light phase and dark phase, respectively. (D) Average activity counts per minute in WT and Gipr$^{-/-}$ mice. $n = 5$ mice/group. $^{**}P < 0.01$, 10-week-old vs 50-week-old mice, $^{\#}P < 0.05$; $^{\#\#}P < 0.01$, WT vs Gipr$^{-/-}$ mice.

(Fig. 4D). However, BT in dark phase (Fig. S1(C) and S1(D)) and HR in both light and dark phases (Fig. S1(E) and S1(F)), measured simultaneously, were paradoxically decreased in Gipr$^{-/-}$ mice compared with WT mice despite increased physical activity.

Discussion

GIP was originally designated gastric inhibitory polypeptide for its influence on gastric acid secretion, and was later designated glucose-dependent insulinotropic polypeptide for its stimulation of insulin secretion from pancreatic β-cells. Studies using Gipr$^{-/-}$ mice have shown that GIP also has physiological roles in fat accumulation into adipose tissues [9] and calcium accumulation into bone [15], and thus a more appropriate referent of the acronym, gut-derived nutrient-intake polypeptide, has been recently proposed to more accurately reflect its physiological function [16]. The importance of inhibition of GIP signaling on fat accumulation *in vivo* was first demonstrated by Miyawaki et al., who found that male Gipr$^{-/-}$ mice fed a high-fat diet exhibit dramatically lesser adiposity than WT mice [9]. In addition, Hansotia et al. showed that single incretin (either GIP or glucagon-like peptide-1, GLP-1) receptor knockout mice as well as double incretin (both GIP and GLP-1) receptor knockout mice exhibited reduced body weight gain and adipose tissue accretion after a 20-week high-fat diet [17].

In the present study, we clearly show that 50-week-old Gipr$^{-/-}$ mice on a normal diet had reduced fat mass but sustained lean mass independent of changes in body weight or food intake, while 50-week-old WT mice showed dramatically increased fat mass and decreased lean mass, a characteristic of aging-associated changes in body composition. In young mice on normal diet, Xie et al. reported that the percentage of total fat was significantly increased and the amount of lean mass was reduced in 1-month-old and 5-month-old Gipr$^{-/-}$ females [18], however their results and our results are incommensurable because of the differences in sex, age, and breeding environments.

We also demonstrated that such alterations in body composition protected Gipr$^{-/-}$ mice from the development of insulin resistance (Fig. 3B). In C57BL/6 mice on a normal diet, glucose tolerance itself does not necessarily deteriorate with age because glucose-stimulated insulin secretion increases to compensate for age-related insulin resistance [19]. In our study, aged WT mice retained normal glucose tolerance, although a compensatory increase in insulin secretion was required to achieve the same blood glucose levels as in young mice. On the other hand, aged Gipr$^{-/-}$ mice showed better glucose tolerance during OGTT than in young Gipr$^{-/-}$ mice without incremental insulin secretion. Although Gipr$^{-/-}$ mice showed mild hyperglycemia compared with WT mice at early phases after oral glucose challenge, overall glycemic control as shown by HbA_{1c} was not worse in Gipr$^{-/-}$ mice. Moreover, the insulin secretory response to glucose and GLP-1 stimulation was intact in the islets of Gipr$^{-/-}$ mice compared with those of WT controls, indicating that β-cell function was not impaired by long-term inhibition of GIP signaling. We therefore conclude that aged Gipr$^{-/-}$ mice are protected from the development of aging-associated insulin resistance, which is associated with amelioration in glucose tolerance.

Stability of lean mass is another most important anti-aging phenomenon observed in aged Gipr$^{-/-}$ mice. Skeletal muscle accounts for more than half (~55%) of total lean mass, and maintenance of skeletal muscle mass is important for its metabolic quality as well as physical strength and functional status. There is an ongoing reduction of skeletal muscle mass in weight-stable elderly men and women [4,5], suggesting that weight stability in older individuals does not imply body composition stability, however, Gipr$^{-/-}$ mice gained lean mass with age, maintaining the same percentage of lean mass as the young mice (Fig. 2G). Favorable body composition with decreased fat mass and sustained lean mass can be promoted by physical exercise [20]. Our results clearly show that Gipr$^{-/-}$ mice are spontaneously hyperactive (Fig. 3C and D), which may contribute to favorable body composition and improved insulin sensitivity in older age. Surprisingly, BT and HR measured simultaneously were decreased in Gipr$^{-/-}$ mice compared with WT mice despite increased physical activity, characteristics resembling the physiological changes that take place in long-lived calorie restricted animals [21–23].

In conclusion, we have demonstrated that long-term inhibition of GIP signaling prevents development of aging-associated insulin resistance through body composition changes. Considering the difficulty of maintaining dietary restriction and exercise training for a prolonged period, GIP antagonism might be considered for further investigation as a therapeutic option against metabolic disorders related to aging.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2007.09.128.

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