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Absence of GIP secretion alleviates age-related obesity and insulin resistance

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Abstract

Glucose-dependent insulinotropic polypeptide (GIP) is an incretin secreted from enteroendocine K cells after nutrient ingestion. Fat strongly induces GIP secretion, and GIP hypersecretion is involved in high-fat diet-induced obesity and insulin resistance. Aging also induces GIP hypersecretion, but its effect on body weight gain and insulin sensitivity remains unclear. In the present study, we investigated the effect of GIP on age-related body weight gain and insulin resistance using GIP-knockout homozygous ($GIP^{-/-}$) and heterozygous ($GIP^{+/-}$) mice, which have entirely absent and 50% reduced GIP secretion compared to wild-type (WT) mice, respectively. Under 12% fat-containing normal diet feeding condition, body weight was significantly lower in $GIP^{-/-}$ mice compared to that in WT and $GIP^{+/-}$ mice from 38 weeks of age, while there was no significant difference between WT and $GIP^{+/-}$ mice. Visceral and subcutaneous fat mass were also significantly lower in $GIP^{-/-}$ mice compared to those in WT and $GIP^{+/-}$ mice. During oral glucose tolerance test, blood glucose levels did not differ among the three groups. Insulin levels were significantly lower in $GIP^{-/-}$ mice than those in WT and $GIP^{+/-}$ mice. During insulin tolerance test, $GIP^{-/-}$ mice showed higher insulin sensitivity than that of WT and $GIP^{+/-}$ mice. Adiponectin mRNA levels were increased and leptin mRNA levels tended to be decreased in adipose tissue of $GIP^{-/-}$ mice. These results demonstrate that GIP is involved in age-related obesity and insulin resistance and that inhibition of GIP secretion alleviates age-related fat mass gain and insulin resistance under carbohydrate-based diet feeding condition.

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Introduction

Life expectancy has increased in developed countries, and is accompanied by the main age-related changes in body composition, which are an increase in fat mass and a decrease in muscle mass. In addition, visceral fat accumulation causes insulin resistance through inflammation (Kalyani et al 2014). This age-related change is called “Sarcopenic obesity,” and is an important health issue in aging societies (Prado et al 2012, Cleasy et al 2016). Obesity is related to a decline of activities of daily living (ADL) in elderly people, and is also related to the high prevalence of medical disorders such as diabetes, hyperlipidemia, and hypertension (Cheng at al 2013, Chang et al 2017). It is therefore important for elderly people to prevent excessive fat accumulation with aging.

Glucose-dependent insulintropic polypeptide/gastric inhibitory polypeptide (GIP) is an incretin secreted from enteroendocrine K cells in response to glucose and fat ingestion, and enhances glucose-dependent insulin secretion through the GIP receptor (GIPR) expressed in pancreatic β-cells (Seino at al 2013). GIPR is expressed in adipose tissue as well (Joo et al 2017). GIP plays an important role in high-fat diet (HFD)-induced obesity and insulin resistance (Harada et al 2008, Joo et al 2017). Previous studies using GIP immunoneutralization, GIPR-knockout mice, and GIPR antagonists reported that inhibition of GIP signaling ameliorates HFD-induced obesity and insulin resistance (Miyawaki et al 2002, McClean at al 2008, Boylan et al 2015). HFD strongly stimulates GIP secretion (Iwasaki et al 2015, Sankoda et al 2017, Murata et al 2019); and inhibition of GIP secretion also alleviates HFD-induced obesity and insulin resistance (Nasteska et al 2014). Previous human study found that GIP secretion after glucose ingestion is increased in elderly subjects (Garduno-Garcia et al 2018). We previously reported that aged

mice exhibit not only GIP hypersecretion but also excessive fat mass and insulin resistance under normal diet feeding condition (Ikeguchi et al 2018). These results indicate that GIP hypersecretion from K cells may be involved in age-related fat mass gain and insulin resistance. However, the effect of age-related GIP hypersecretion on body weight and fat mass gain, and insulin resistance remains unclear. In this study, we investigated the effect of entirely absent and 50% reduced GIP secretion on age-related body weight, body fat composition, glucose tolerance, and insulin sensitivity under carbohydrate-based normal diet feeding condition using GIP-knockout mice.

Materials and Methods

Animals

GIP-knockout mice were generated previously (Nasteska et al 2014). GIP secretion was entirely absent in homozygous ($GIP^{-/-}$) mice, and was reduced in heterozygous ($GIP^{+/-}$) mice by 50% compared with that in wild-type (WT) mice, respectively. Male $GIP^{-/-}$, $GIP^{+/-}$ and WT littermate mice were used in all experiments. Aged mice were defined as age one year (50-60 weeks) as described previously (Ikeguchi et al 2018). Experiments were carried out in three separate cohorts, each consisting of three groups of five to seven mice. Body weight and fat weight were evaluated and oral glucose tolerance test (OGTT) was performed in the first cohort. Blood samples under non-fasting condition were collected, and body fat composition analyzed by computed tomography (CT) scan, locomotor activity, insulin sensitivity determined by insulin tolerance test (ITT), and measurement of GIP and GLP-1 content in intestine were evaluated in the second cohort. Energy expenditure, food intake, and gene expression were evaluated in the

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third cohort. The mice were housed in an air-controlled 25°C room with a dark-light cycle of 10 and 14 hr with free access to water and normal diet food (3.73kcal/g; 12% fat, 23% protein, and 65% carbohydrate; Funabashi Farm, Funabashi, Japan). Animal care and procedures were approved by Kyoto University Animal Care Committee (MedKyo15298).

Blood samples

50 µl blood samples were collected from the tail vein at 10:00 a.m. under non-fasting condition. After a 16-hr fasting period, OGTTs (2g glucose/body weight [kg] for blood glucose levels, plasma insulin and total GIP levels, and 6g glucose/body weight [kg] for plasma glucagon-like peptide-1 [GLP-1] levels) were performed. Blood samples were collected from the tail vein at 0, 15, 30, 60, and 120 min after oral glucose administration by oral gavage. Blood glucose levels were measured by the glucose oxidase method (Sanwa Kagaku Kenkyusho, Nagoya, Japan). Plasma insulin, total GIP levels, and GLP-1 levels were measured by insulin ELISA kit (Shibayagi, Gunma, Japan), total GIP ELISA kit (Millipore Corporation, Billerica, MA), and V-PLEX GLP-1 Total Kit (MESO SCALE DISCOVERY, Rockville, MD), respectively. For ITT, human regular insulin (Eli Lilly and Company, Indianapolis, IN) at a dose of 0.75 U insulin/body weight (kg) was injected to the intraperitoneal cavity after a 4-hr fasting period. Blood glucose levels were measured at 0, 30, 60, 90, and 120 min after insulin administration.

GIP and GLP-1 content in intestine

Small intestine and colon (4 cm length) were taken from the mice. Samples were extracted with 1 mL of 0.2N perchloric acid and were centrifuged for 15 minutes at 15000 rpm at 4° C. The

supernatant was used for measurement of GIP and GLP-1 content in intestine as previously described (Ikeguchi et al 2018).

Measurement of body fat composition

After the mice were dissected, the weights of visceral fat (both sides of epididymal fat), subcutaneous fat (both sides of inguinal subcutaneous fat), and the right side of the gastrocnemius muscle were measured. Body fat mass, lean body mass, and fat content in liver were measured using a La Theta experimental animal computed tomography (CT) scan system (LCT-100M, Hitachi Aloka Medical, Tokyo, Japan). Contiguous 2 mm slice images from the top of the diaphragm to the caudal region were used for quantitative analysis of fat mass and lean body mass (visceral mass without visceral fat mass and subcutaneous fat mass) of each mouse by La Theta software (vs. 3.00). Fat content in the liver was calculated from density data of fat (100 % fat) and muscle (0 % fat).

Energy Expenditure and Locomotor Activity

Energy expenditure was calculated using an Alco System model 2000 (Alco System, Chiba, Japan). Locomotor activity was monitored as distance in a standardized locomotor chamber box (15 × 35 × 40 cm). After the mice were placed into the tracking box for 48 hr, locomotor activity was monitored for 24 hr using SMART Video Tracking System (Panlab SL, Barcelona, Spain) with free access to water and diet.

Quantitative RT-PCR

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Total RNAs of the islets, small intestine, subcutaneous fat, and visceral fat were extracted using RNeasy Mini Kit (Qiagen, Hilden, Germany) and TRIzol Reagant (Invitrogen, Grand Island, NY). For cDNA synthesis, RNA was reverse-transcribed using a PrimeScript RT reagent kit (Takara Bio, Shiga, Japan). The mRNA expression levels were measured by quantitative RT-PCR using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, California, CA). SYBR Green PCR Master Mix (Applied Biosystems) was prepared for the PCR run. β-actin was used as the internal control. Each data point of mRNA expression was standardized against β-actin. Primer pairs for PCR were designed previously (Ikeguchi et al 2018, Joo et al 2017).

Statistical analysis

All data are expressed as the mean ± SE. Statistical analysis was carried out using one-way ANOVA with the Tukey-Kramer multiple comparison tests. *P* values < 0.05 were considered significant.

Results

Effect of absent and reduced GIP secretion on body weight gain with aging

Body weight was significantly decreased in $GIP^{-/-}$ mice compared to that in WT and $GIP^{+/-}$ mice from 38 weeks of age, but there was no difference between WT and $GIP^{+/-}$ mice (Fig. 1A). Non-fasting glucose, total GIP, and insulin levels were measured once each 5 weeks. There was no difference in blood glucose levels (Fig. 1B) among the three groups. Total GIP levels were increased with aging in both WT and $GIP^{+/-}$ mice (Fig. 1C). Total GIP levels were lower in $GIP^{+/-}$ mice compared to those in WT mice. Total GIP levels were not detectable in $GIP^{-/-}$ mice. Insulin

levels were increased with aging in WT and $GIP^{+/-}$ mice (Fig. 1D), but insulin levels were significantly lower in $GIP^{-/-}$ mice compared to those in WT and $GIP^{+/-}$ mice from 25 weeks of age.

Effect of absent and reduced GIP secretion on body fat mass

Subcutaneous and visceral fat weights were significantly lower in $GIP^{-/-}$ mice compared to those in WT and $GIP^{+/-}$ mice (Fig. 2A). There was no significant difference in fat weight between WT and $GIP^{+/-}$ mice. Gastrocnemius muscle and liver weight did not differ among the three groups (Fig. 2B and 2C). CT scan analysis showed that $GIP^{-/-}$ mice had significantly lower body fat mass and fat content in liver compared to those in WT and $GIP^{+/-}$ mice (Fig. 2D and 2E), but there was no significant difference between WT and $GIP^{+/-}$ mice. Lean body mass did not differ among the three groups (Fig. 2F). Energy expenditure was significantly higher in $GIP^{-/-}$ mice compared to that in WT and $GIP^{+/-}$ mice (Fig. 2G), but did not differ between WT and $GIP^{+/-}$ mice. Locomotor activity and food intake did not differ among the three groups (Fig. 2H and 2I).

Effect of absent and reduced GIP secretion on glucose tolerance and insulin sensitivity

During OGTT, blood glucose levels were not significantly different among the three groups (Fig. 3A). Insulin levels were remarkably lower in $GIP^{-/-}$ mice compared to those in WT and $GIP^{+/-}$ mice (Fig. 3B). Insulin levels did not differ between WT and $GIP^{+/-}$ mice. Total GIP levels were reduced and not detectable in $GIP^{+/-}$ and $GIP^{-/-}$ mice, respectively (Fig. 3C). GIP secretion (area under the curve of GIP during OGTT) in $GIP^{+/-}$ mice was reduced by 50% compared to that in WT mice. Total GLP-1 levels at 15 min were significantly lower in $GIP^{-/-}$ mice than those in WT mice (Fig. 3D); total GLP-1 levels did not differ between WT and $GIP^{+/-}$ mice. During ITT, blood glucose

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levels were lower in $GIP^{-/-}$ mice compared with those in WT and $GIP^{+/-}$ mice (Fig. 3E). Insulin sensitivity did not differ between WT and $GIP^{+/-}$ mice. GIP content in small intestine was reduced and not detectable in $GIP^{+/-}$ and $GIP^{-/-}$ mice, respectively (Fig. 3F). GIP content in small intestine was significantly smaller in $GIP^{-/-}$ mice than those in WT and $GIP^{+/-}$ mice. GIP content was not detected in colon in any of the three groups of mice. GLP-1 content was smaller in the small intestine and colon of $GIP^{-/-}$ mice than that in WT and $GIP^{+/-}$ mice (Fig 3G).

Effect of absent and reduced GIP secretion on GIPR mRNA expression

GIPR was reported to be expressed in islets, small intestine, and adipose tissue (Joo et al 2017, Seino et al 2013, Usdin et al 1993), and the expression levels of GIPR mRNA did not differ among the three groups (Fig. 4A). Expression levels of adiponectin mRNA in adipose tissue were significantly higher in $GIP^{-/-}$ mice compared with those in WT and $GIP^{+/-}$ mice (Fig. 4B). Expression levels of leptin mRNA tended to be decreased in $GIP^{-/-}$ mice, but there was no significant difference among the three groups (Fig. 4C).

Discussion

Both insulin and total GIP levels are increased under HFD-induced obese condition (Nasteska et al 2014, Shimazu-Kuwahara et al 2017, Murata et al 2019). We previously investigated the effects of absent and 50% reduced GIP secretion on HFD-induced obesity and insulin resistance using GIP-knockout mice; we found that reduced GIP secretion as well as absent GIP secretion alleviates fat mass gain and insulin resistance under HFD feeding condition (Nasteska et al 2014). These results demonstrate that reduced GIP secretion as well as absent

GIP secretion contribute to alleviation of HFD-induced obesity and insulin resistance. Insulin and total GIP levels are increased under aged condition (Trahair 2012, Ikeguchi et al 2018, Garduno-Garcia 2018). In the present study, the effects of absent and reduced GIP secretion on age-related fat mass gain and insulin resistance under normal fat diet feeding condition were investigated using the same GIP-knockout mouse used in the previous study. We found that GIP secretion is increased with aging in WT and $GIP^{+/-}$ mice, and that absence of GIP secretion as shown in $GIP^{-/-}$ mice alleviates age-related body weight and fat mass gain and insulin resistance, indicating that GIP hypersecretion is associated with age-related body weight gain and insulin resistance under normal fat diet feeding condition. On the other hand, reduction of GIP secretion as shown in $GIP^{+/-}$ mice did not affect body weight and fat mass gain, and insulin resistance under normal fat diet feeding condition. Previous study using GIPR-knockout mice also showed that the effect of GIP signaling on body weight gain and insulin resistance is less under high-carbohydrate diet feeding condition than it is under HFD feeding condition (Maekawa et al 2018). The study suggests that these effects of GIP reflect the different effects of carbohydrate diet and HFD on body weight and insulin sensitivity. HFD induces more body weight gain and fat mass gain than carbohydrate diet, primarily because fat has a higher caloric value than the same amount of carbohydrate (Jois et al 2016). HFD is also associated with unfavorable changes in the type and number of gut bacteria and bile acids composition in the intestine, which induce obesity and insulin resistance (Hildebrandt et al 2009, Islam et al 2011, Yokota et al 2012, Saad et al 2016). In addition, GIP potentiates insulin secretion from pancreatic β-cells as an incretin and thus plays an important role in hyperinsulinemia under HFD-feeding obese condition (Harada et al 2008). GIP also increases IL-6 expression and

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production in adipocytes in the presence of TNF-α, which is induced by obesity, and enhances HFD-induced insulin resistance through IL-6 signaling (Joo et al 2017). These findings demonstrate that the obesity and insulin resistance under HFD feeding condition are accelerated by GIP signaling compared to those under carbohydrate-based normal diet feeding condition. Thus, we might well not be able to find a major difference in body weight and insulin sensitivity between WT and $GIP^{+/-}$ mice under normal fat diet feeding condition used in this study.

It was previously reported that aged GIPR-knockout mice show improvement of insulin resistance and reduced fat mass without a reduction of body weight compared to aged WT mice under normal diet feeding condition (Yamada et al 2007). Locomotor activity and lean body mass were increased and body temperature and heart rate were decreased in these aged GIPR-knockout mice. However, in the present study, locomotor activity, lean body mass, and rectal temperature (data not shown) did not differ in WT and $GIP^{-/-}$ mice. There are several possible explanations for the difference between the results from the GIPR-knockout mice and our $GIP^{-/-}$ mice. First, the methods of measurement of locomotor activity and body temperature in our study are quite different from those in the previous study. Second, the measurement area used to evaluate fat and lean body mass by CT scan differs between the previous study and our study. Third, housing conditions and mouse microbiota might contribute to the differences in body weight gain. Therefore, a direct comparison study is needed to clarify the difference of phenotype between aged GIPR-knockout and $GIP^{-/-}$ mice.

Muscle and liver have much higher contribution to glucose uptake and energy expenditure than adipose tissue (DeFronzo et al 1987, Gallagher et al 1998). Lean body mass

should be considered in the study of glucose uptake and energy expenditure. In this study, however, lean body mass was measured by CT scan from the top of the diaphragm to the femoral head. This area mass reflects the weight of internal organs including iliopsoas muscles but not the muscle weight of whole body. Therefore, we performed OGTT in proportion to whole body and calculated energy expenditure by the whole body weight.

Recently, antagonism of the GIP receptor has come to be seen as potential therapeutic target for obesity and insulin resistance (McClean et al 2007, McClean et al 2008, Boylan et al 2015, Kaneko et al 2019). In this study, GIP is shown to be involved in age-related obesity and insulin resistance under normal fat diet feeding condition. Furthermore, inhibition of GIP secretion might enable us to prevent fat accumulation with aging and declination of ADL levels in the elderly. Interestingly, inhibition of GIP secretion might also have a favorable effect on extension of lifespan; a recent study using GIPR-knockout mice reported that inhibition of GIP signaling is involved in extension of lifespan (Hoizumi et al 2019).

In conclusion, GIP is involved in age-related obesity and insulin resistance, and inhibition of GIP secretion alleviates age-related body weight and fat mass gain, and insulin resistance under carbohydrate-based feeding condition.

Author contributions

Y.K. and N.H. planned the study, researched data, contributed to discussion, wrote, reviewed and edited the manuscript. S.K-S. planned the study and researched data. S.Y., E.I., Y.M., S.K., and T.H. researched data. N.I. planned the study, contributed to discussion, and edited the manuscript. All authors approved the final version of the manuscript.

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Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research.

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Figure legends

Fig 1. Body weight gain and non-fasting blood glucose levels, GIP levels, and insulin levels in GIP$^{-/-}$ and GIP$^{+/-}$ mice

(A) Body weight, (B) blood glucose levels, (C) total GIP levels, and (D) insulin levels (n = 6-7). WT mice (white circles), GIP$^{+/-}$ mice (gray circles), and GIP$^{-/-}$ mice (black circles).

***P<0.001 vs. WT mice. $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. GIP$^{+/-}$ mice.

Fig 2. Body composition, energy expenditure, locomotor activity, and food intake in GIP$^{-/-}$ and GIP$^{+/-}$ mice

(A) Subcutaneous and visceral fat weight, (B) gastrocnemius muscle weight, and (C) Liver weight in 60 weeks old mice (n = 5-6). (D) Subcutaneous and visceral fat mass, (E) lean mass, and (F) fat content in liver estimated by CT scan in 58 weeks old mice (n = 5-6). Pink, yellow, and blue areas represent visceral fat, subcutaneous fat, and lean mass, respectively. (G) Energy expenditure in 51 weeks old mice (n = 6). (H) Locomotor activity in 54 weeks old mice (n = 6). (I) Food intake in 52 weeks old mice (n = 6). WT mice (white bars), GIP$^{+/-}$ mice (gray bars), and GIP$^{-/-}$ mice (black bars). *P<0.05, **P<0.01, ***P<0.001. n.s.; no significant difference.

Fig 3. Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT)

(A) Blood glucose levels, (B) insulin levels, and (C) total GIP levels during OGTT (2 g glucose/kg body weight) in 52 weeks old mice (n=6). (D) Total GLP-1 levels during OGTT (6 g glucose/kg body weight) in 54 weeks old mice (n=6). (E) Blood glucose levels during ITT (0.75U insulin/kg body weight) in 54 weeks old mice. (F) GIP content in small intestine and colon in GIP$^{-/-}$ and

390 $GIP^{+/-}$ in 54 weeks old mice (n=6). (G) GLP-1 content in small intestine and colon in $GIP^{-/-}$ and
391 $GIP^{+/-}$ in 54 weeks old mice (n=6). WT mice (white bars and circles), $GIP^{+/-}$ mice (gray bars and
392 circles), and $GIP^{-/-}$ mice (black bars and circles). *P<0.05, **P<0.01, ***P<0.001 vs. WT mice.
393 $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. $GIP^{+/-}$ mice. n.s.; no significant difference. AUC; area under the
394 curve.
395
396 **Fig 4. Gene expression in islets, upper small intestine, and adipose tissue in $GIP^{-/-}$ and $GIP^{+/-}$**
397 **mice**
398 (A) Expression levels of GIPR mRNA in islets, upper small intestine, subcutaneous fat, and
399 visceral fat in 54 weeks old mice (n = 6). Expression levels of (B) Adiponectin mRNA and (C)
400 Leptin mRNA expression in visceral fat in 54 weeks old mice (n = 6). WT mice (white bars), $GIP^{+/-}$
401 mice (gray bars), and $GIP^{-/-}$ mice (black bars). *****P<0.05 and **P<0.01. n.s.; no significant
402 difference.

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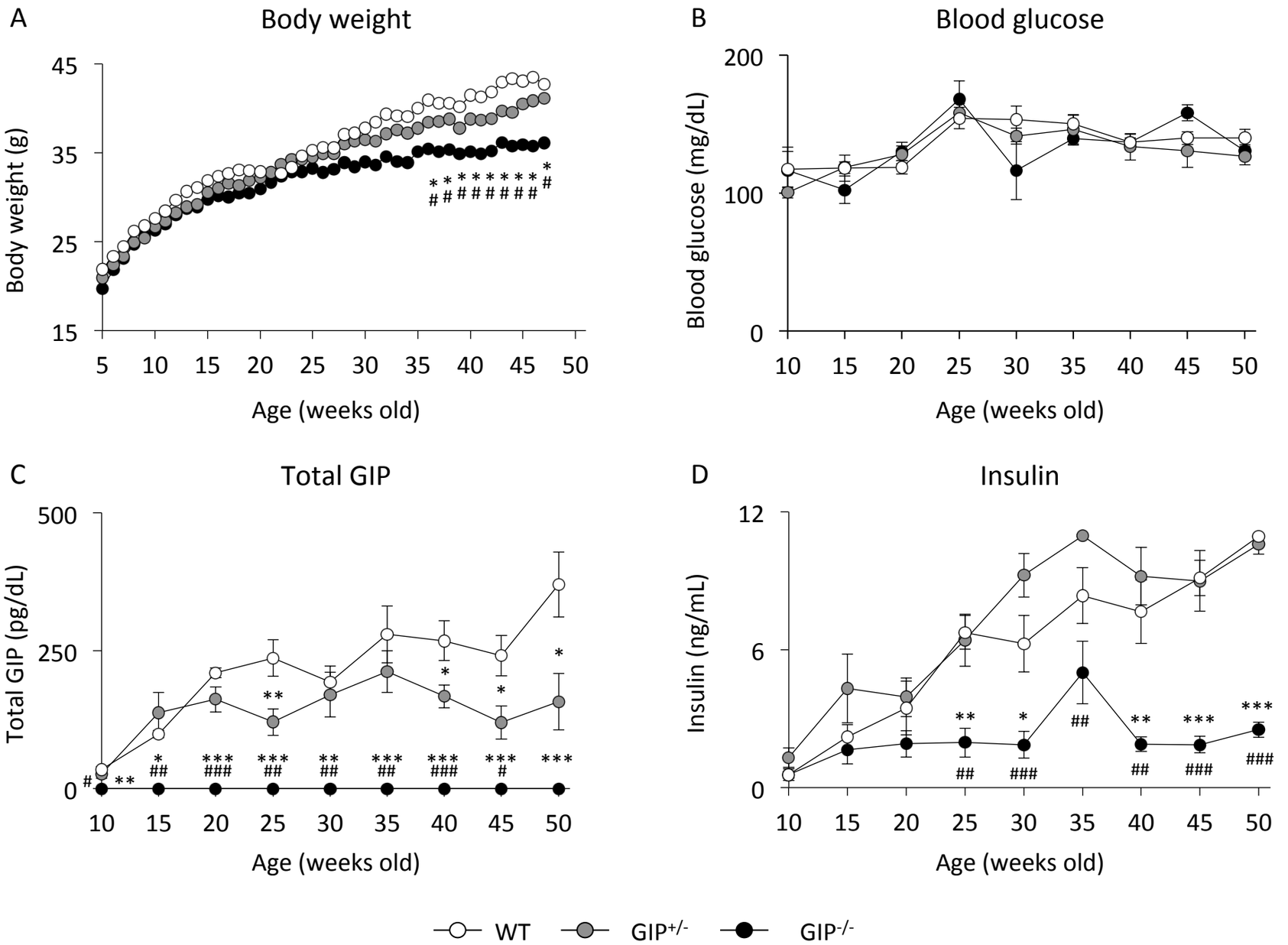
Fig. 1



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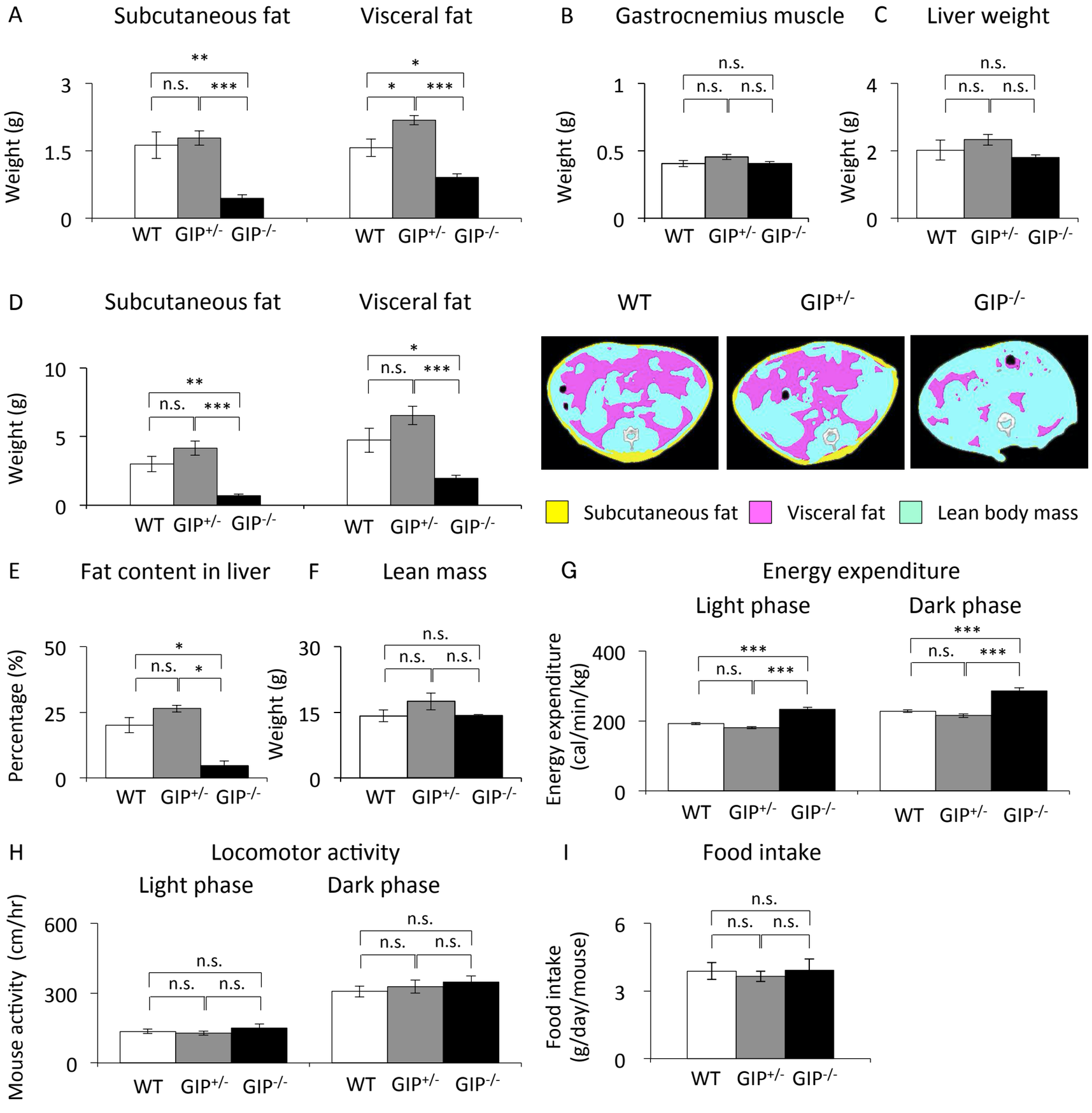

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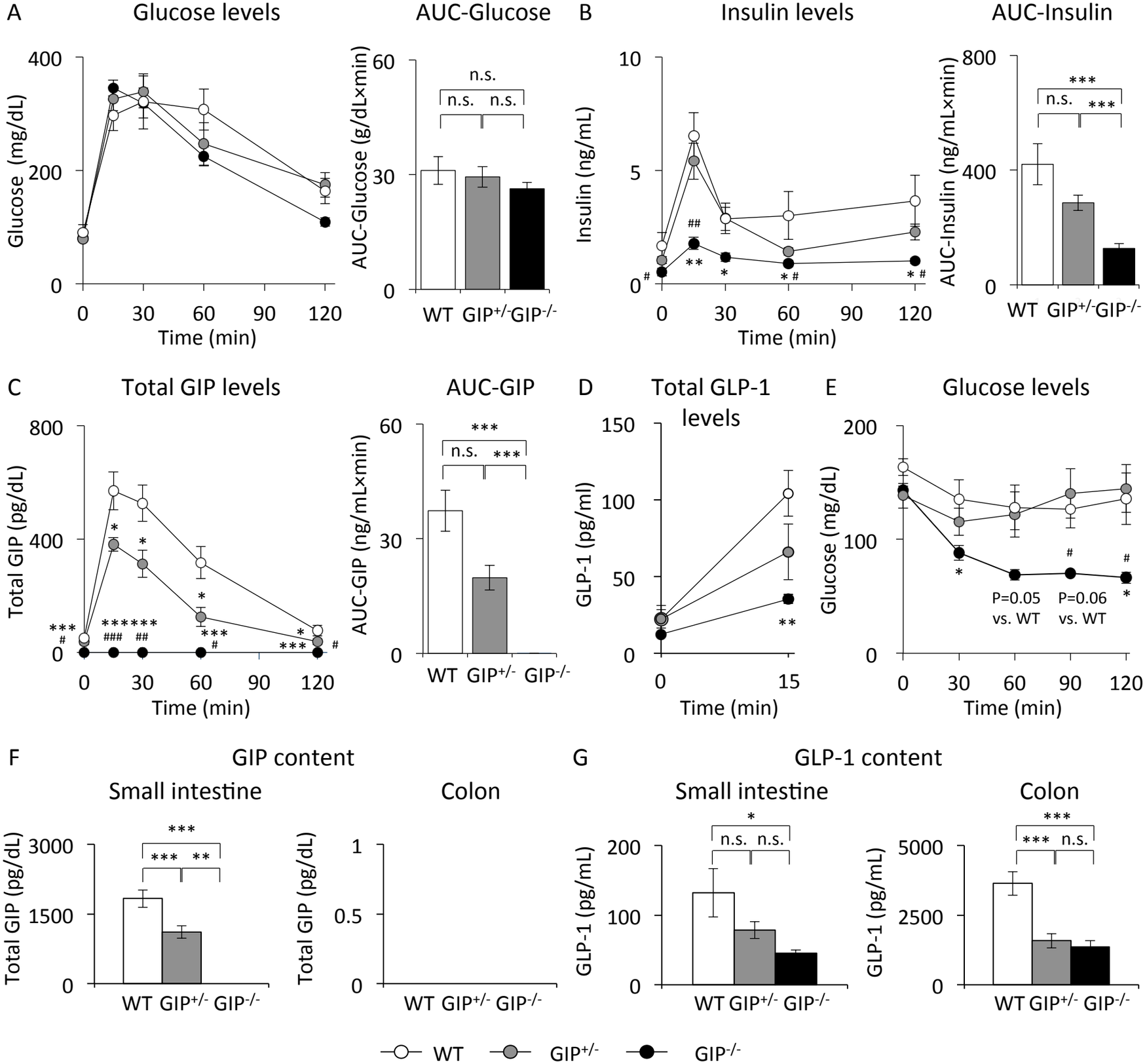
Fig. 3



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