Am J Physiol Endocrinol Metab 317: E53–E64, 2019.
First published April 16, 2019; doi:10.1152/ajpendo.00200.2018.

RESEARCH ARTICLE

Medium-chain triglyceride diet stimulates less GIP secretion and suppresses body weight and fat mass gain compared with long-chain triglyceride diet

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Submitted 21 May 2018; accepted in final form 11 April 2019

Murata Y, Harada N, Yamane S, Iwasaki K, Ikeguchi E, Kanemaru Y, Harada T, Sankoda A, Shimazu-Kuwahara S, Joo E, Poudyal H, Inagaki N. Medium-chain triglyceride diet stimulates less GIP secretion and suppresses body weight and fat mass gain compared with long-chain triglyceride diet. *Am J Physiol Endocrinol Metab* 317: E53–E64, 2019. First published April 16, 2019; doi: 10.1152/ajpendo.00200.2018.—Gastric inhibitory polypeptide (GIP) is an incretin secreted from enteroendocrine K cells and potentiates insulin secretion from pancreatic β-cells. GIP also enhances long-chain triglyceride (LCT) diet-induced obesity and insulin resistance. Long-term intake of medium-chain triglyceride (MCT) diet is known to induce less body weight and fat mass gain than that of LCT diet. However, the effect of MCT diet feeding on GIP secretion and the effect of GIP on body weight and fat mass under MCT diet-feeding condition are unknown. In this study, we evaluated the effect of single MCT oil administration on GIP secretion and compared the effect of long-term MCT and LCT diet on body weight and fat mass gain in wild-type (WT) and GIP-knockout (GIP KO) mice. Single administration of LCT oil induced GIP secretion but that of MCT oil did not in WT mice. Long-term intake of LCT diet induced GIP hypersecretion and significant body weight and fat mass gain compared with that of control fat (CF) diet in WT mice. In contrast, MCT diet did not induce GIP hypersecretion, and MCT diet-fed mice showed smaller increase in body weight and fat mass gain compared with CF diet-fed mice. In GIP KO mice, body weight and fat mass were markedly attenuated in LCT diet-fed mice but not in MCT diet-fed mice. Our results suggest that long-term intake of MCT diet stimulates less GIP secretion and suppresses body weight and fat mass gain compared with that of LCT diet.

gastric inhibitory polypeptide; incretin; long-chain triglyceride; medium-chain triglyceride; obesity

INTRODUCTION

Obesity is characterized by an excess of body fat and is one of the risk factors of lifestyle-related diseases such as type 2 diabetes, heart disease, stroke, hypertension, and cancer (1, 53). A high-fat diet (HFD) is involved in the increasing prevalence of obesity, mainly due to its high energy density (40).

Most of the fat in a typical diet consists of long-chain triglycerides (LCTs) that are made up of three long-chain fatty acids (>C14) and glycerol. Excessive intake of LCT diet induces obesity (8). However, differences in fatty acid composition of dietary fat other than energy density may have distinct physiological effects on body weight and body fat gain (35). Coconut oil and some parenteral nutrition formulas contain large amounts of medium-chain triglycerides (MCTs) (35). MCT consists of three medium-chain fatty acids (MCFAs; C6-C12) and glycerol. In contrast to LCT, recent evidence suggests that MCT may be useful in preventing metabolic diseases (35, 47). A meta-analysis has shown that MCT diet induces less body weight and fat mass gain than LCT diet (32). Numerous mechanisms for decreased adiposity in response to MCT diet have been proposed, including improvement of lipid metabolism, activation of fatty acid oxidation in the liver, increased energy expenditure, and changes in the intestinal bacterial flora in animal (44, 52, 57) and human (36, 46, 48) studies. Additionally, some studies have shown that MCT may downregulate the production of inflammatory cytokines (10, 28) and attenuate insulin resistance (15, 16).

Glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide/glucose-dependent insulinotropic polypeptide (GIP) are two major incretins secreted from the intestine in response to meal ingestion (22, 41) and potentiate glucose-dependent insulin secretion from pancreatic β-cells. GLP-1 and GIP also play a role in the pathophysiology of obesity in response to energy-dense diets (9). GLP-1 is secreted from enteroendocrine L cells located in the small intestine and colon in response to various nutrients including dietary fat (41, 51), and promotes satiety, decreases energy intake, and induces weight loss (4). In contrast, GIP is secreted from enteroendocrine K cells located in the small intestine and enhances LCT diet-induced lipid accumulation and insulin resistance (12, 20). Previous studies using a GIP antagonist (14, 18, 29), GIP-specific neutralizing antibody (7), and GIP receptor-knockout (GIPR KO) mice (30) have shown that inhibition of GIPR signaling ameliorates LCT diet-induced obesity and insulin resistance. Our previous study showed that GIP KO mice gain less body weight and body fat mass compared with WT mice under LCT diet feeding, resulting in attenuation of insulin resistance (33). These results suggest that GIP signaling is a key mediator of LCT diet-induced obesity and insulin resistance (21, 43).

The MCT diet has been shown to ameliorate metabolic health, but the effects of long-term intake of MCT diet on GIP secretion and the effect of GIP on body weight and body fat mass under the MCT diet-feeding condition are unknown. In this study, we evaluated body weight, fat mass, and insulin

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sensitivity under the long-term MCT diet-feeding condition in WT mice and clarified the role of GIP using GIP KO mice, which do not secrete GIP.

MATERIAL AND METHODS

Animals. Male WT and GIP KO mice (C57BL/6J background) were maintained under conditions of a 14:10-h light-dark cycle with free access to water and food. WT mice 13–17 wk old were used for oral oil tolerance tests. GIP KO mice were generated as described previously (33). At 6 wk old, WT and GIP KO mice were divided into the following three groups: control fat (CF; 10% fat by energy, 3.85 kcal/g) diet, high LCT (LCT; 45% fat by energy, 4.73 kcal/g) diet, and high MCT (MCT; 45% fat by energy, 4.73 kcal/g) diet (Research Diets, New Brunswick, NJ). The compositions of the experimental diets are listed in Table 1. We conducted three cohort studies. Body weight was measured in all cohorts. Real-time RT-PCR analysis was performed in *cohort 3*, and other experiments were performed in *cohort 1*. Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) were performed at 13 and 20 wk of feeding, respectively. Fat mass, energy expenditure, and locomotor activity were measured at 24–26 wk of diet feeding. Real-time quantitative RT-PCR analysis was performed at 24 wk of diet feeding. Animal care and procedures were approved by the Kyoto University Animal Care Committee (MedKyo 16584).

Measurement of glucose, insulin, total GIP, and total GLP-1 levels. Blood glucose levels were measured by glucose oxidase method (Sanwa Kagaku Kenkyusho, Nagoya, Japan). Plasma insulin, total GIP, and total GLP-1 levels were measured by insulin enzyme-linked immunosorbent assay (ELISA) kit (Shibayagi, Gunma, Japan), total GIP ELISA kit (Millipore, Billerica, MA), and GLP-1 ELISA kit (Meso Scale Discovery, Rockville, MD), respectively.

Oral oil tolerance test. After 16 h of fasting, oral oil tolerance tests were performed using lard oil (for LCT oil) or MCT oil. MCT oil is composed of 75% caprylic (C8:0) and 25% capric (C10:0) fatty acids. The oils in high dose (10 ml/kg body wt) or low dose (3.3 ml/kg body wt) were administered to WT mice. Sixty microliters of blood samples were collected from the tail vein at 0, 30, and 120 min, respectively, after oral oil administration, and levels of blood glucose, plasma insulin, plasma total GIP, and plasma total GLP-1 were measured.

OGTT and ITT. OGTTs (2 g/kg body wt) were performed after 16 h of fasting. Thirty microliters of blood samples were collected from the tail vein at 0, 15, 30, 60, and 120 min after glucose administration. In ITT, human insulin (100 U/ml; Eli Lilly, Indianapolis, IN) at a dose of 0.5 U/kg body wt was administered intraperitoneally after 4 h of fasting. Blood glucose levels were measured at 0, 30, 60, 90, and 120 min after injection of insulin. Results of ITT are expressed as a percentage of initial blood glucose levels.

Measurement of body fat by computerized tomography scan. Mice were anaesthetized with pentobarbital, fixed in a chamber, and scanned using a La Theta (LCT-100M) experimental animal computerized tomography system (Hitachi Aloka Medical, Tokyo, Japan). Contiguous 2-mm slice images from the diaphragm to the base of the tail were used for quantitative analysis of fat volume by La Theta 1.00 software.

Measurement of energy expenditure and locomotor activity. Energy expenditure and locomotor activity were measured by ARCO 2000 (ARCO System, Chiba, Japan). Mice were housed in individual chambers with free access to water and diet. Energy expenditure and locomotor activity were measured every 5 min over 24 h.

Real-time quantitative RT-PCR analysis. Total RNA was extracted from visceral fat using TRIzol reagent (Invitrogen, Grand Island, NY), and 1 μg total RNA was reverse transcribed using a PrimeScript RT reagent kit (Takara Bio, Shiga, Japan) for cDNA synthesis. SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) was applied for quantitative real-time PCR using an ABI StepOnePlus Real-Time PCR System (Applied Biosystems). The signals of the products were standardized against β-actin signals. Primer sequences for hormone-sensitive lipase (HSL) were 5′-AGACCACATCGCCCACA-3′ and 5′-CCTTTATTGTCAGCTTCTTCAAGG-3′. Others were previously described (21).

Statistical analysis. Results are expressed as the means ± SE. Statistical analysis was performed using Student *t*-test and one-way ANOVA with Tukey's test using SPSS Statistics 20.0 software (IBM, Armonk, NY). $P < 0.05$ was considered statistically significant.

Table 1. *Three types of diet*

	CF Diet	MCT Diet	LCT Diet
Nutrient Composition and Energy			
Protein, %	20	20	20
Carbohydrate, %	70	35	35
Fat, %	10 (4% Lard oil)	45 (39% MCT oil)	45 (39% Lard oil)
Energy, kcal/g	3.85	4.73	4.73
Ingredients, g/100 g			
Casein, 80 mesh	19.0	23.3	23.3
L-cystine	0.3	0.3	0.3
Corn starch	42.9	8.5	8.5
Maltodextrin 10	7.1	11.7	11.7
Sucrose	16.4	20.2	20.2
Cellulose, BW200	4.7	5.8	5.8
Vitamin mix	1.0	1.4	1.4
Mineral mix	4.3	5.2	5.2
Soy bean oil	2.4	2.9	2.9
Lard	1.9	20.7	0
MCT oil	0	0	20.7

CF, control fat; MCT, medium-chain triglyceride; LCT, long-chain triglyceride.

RESULTS

Total GLP-1 and GIP levels after single administration of MCT oil. Previous studies showed that MCT induces GLP-1 secretion in human (11, 27) and that MCT/MCFA does not induce GIP secretion in either humans or leptin-deficient obese mice (19, 25, 38). In this study, we evaluated incretin secretion after single administration of LCT oil or MCT oil in lean C57/BL6J mice. The areas under the curve of (AUCs) blood glucose and plasma insulin levels were not significantly different between the low and high-dose groups after either LCT oil or MCT oil administration (Fig. 1, *A–D*). Total GIP levels were increased after LCT oil administration at the low and high doses (Fig. 1*E*). GIP levels at 30 and 120 min and AUC GIP were significantly higher in the high-dose LCT group than those in the low-dose LCT group. In contrast, GIP levels were not increased after administration of MCT oil in both low- and high-dose groups (Fig. 1*F*). High-dose LCT oil increased total GLP-1 levels but low-dose LCT oil did not (Fig. 1*G*). High-dose MCT oil also increased GLP-1 levels with a peak at 120 min, but there was no difference in AUC GLP-1 between the low- and high-dose groups after MCT oil administration (Fig. 1*H*). These results indicate that MCT oil stimulates GLP-1 secretion but not GIP secretion in lean mice.

Effect of MCT diet on body weight and body fat mass in WT and GIP KO mice. In *cohort 1*, LCT diet induced significant body weight gain compared with CF diet in WT mice and the body weight of the LCT diet-fed group was 47.0% higher than that of the CF diet-fed group after 24-wk diet administration (Fig. 2*A*). MCT diet also induced body weight gain, and the

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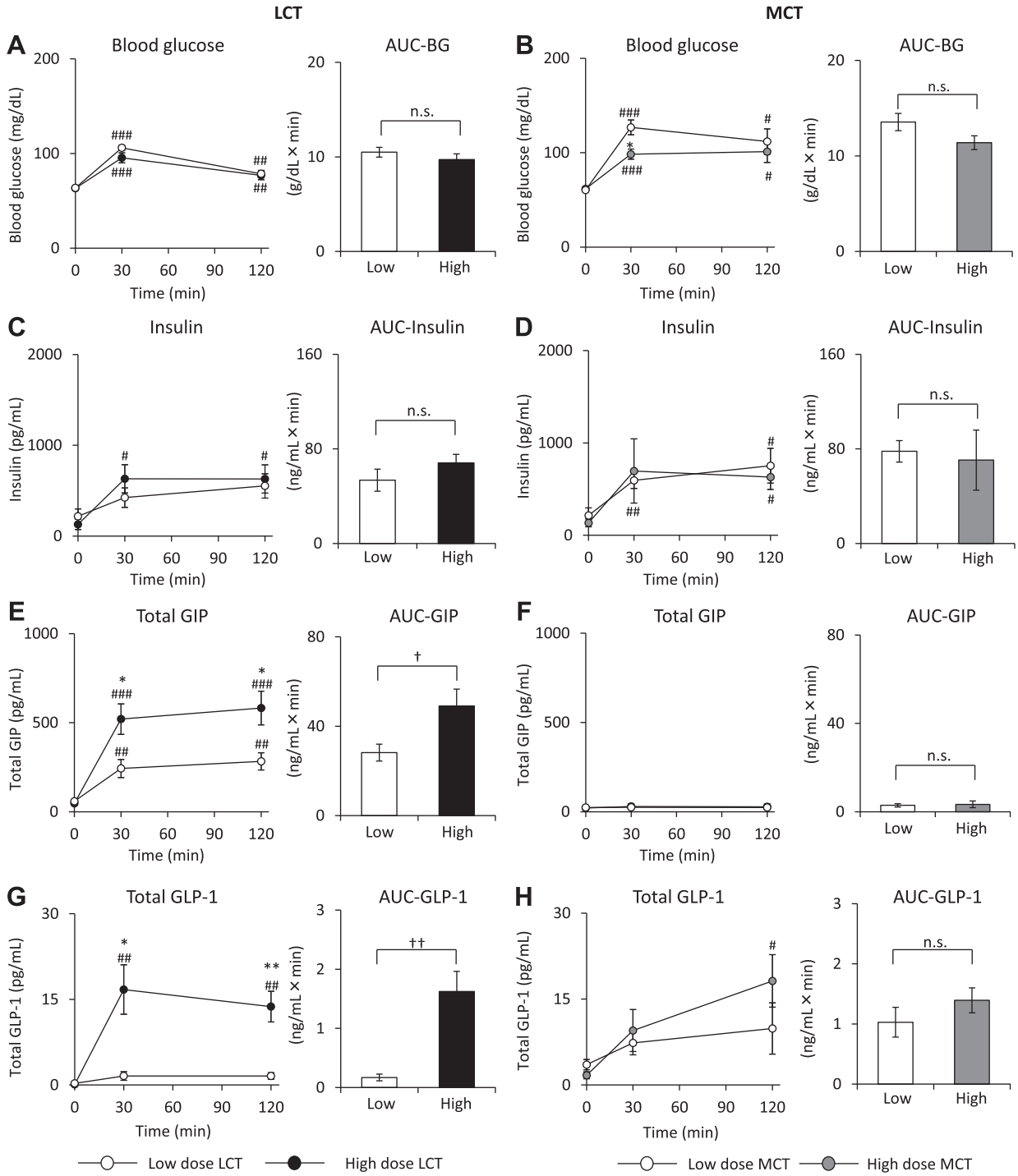

Fig. 1. Blood glucose, insulin, and incretin levels after single administration of long-chain triglyceride (LCT) or medium-chain triglyceride (MCT) oil. Blood glucose (BG) (*A* and *B*), plasma insulin (*C* and *D*), plasma total gastric inhibitory polypeptide (GIP) (*E* and *F*), and plasma total glucagon-like peptide-1 (GLP-1; *G* and *H*) levels during oral administration of LCT oil (*A*, *C*, *E*, and *G*) or MCT oil (*B*, *D*, *F*, and *H*) ($n = 6$). Low dose of oil administration group (3.3 ml/kg) is represented by white circles and bars. High dose of LCT oil administration group is represented by black circles and bars. High dose of MCT oil administration group is represented by gray circles and bars. AUC, area under the curve. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. 0 min (Student *t*-test). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. low-dose group (Student *t*-test). $\dagger P < 0.05$, $\dagger\dagger P < 0.01$ (Student *t*-test); n.s. no significance.

body weight of MCT diet-fed group was 10.7% higher than that of the CF diet-fed group after 24-wk diet administration. Accordingly, weight gain of the MCT diet-induced group was significantly less than that of the LCT diet-induced group at the end of the 24-wk diet administration. In GIP KO mice, both LCT diet and MCT diet increased body weight over 24 wk. Th body weights of LCT diet and MCT diet-fed mice were 28.1 and 7.5% higher than those of CF diet-fed mice, respectively, after 24-wk diet administration (Fig. 2*B*). In *cohorts 2* and *3*, the body weight of the LCT diet-fed group was 60.9% and

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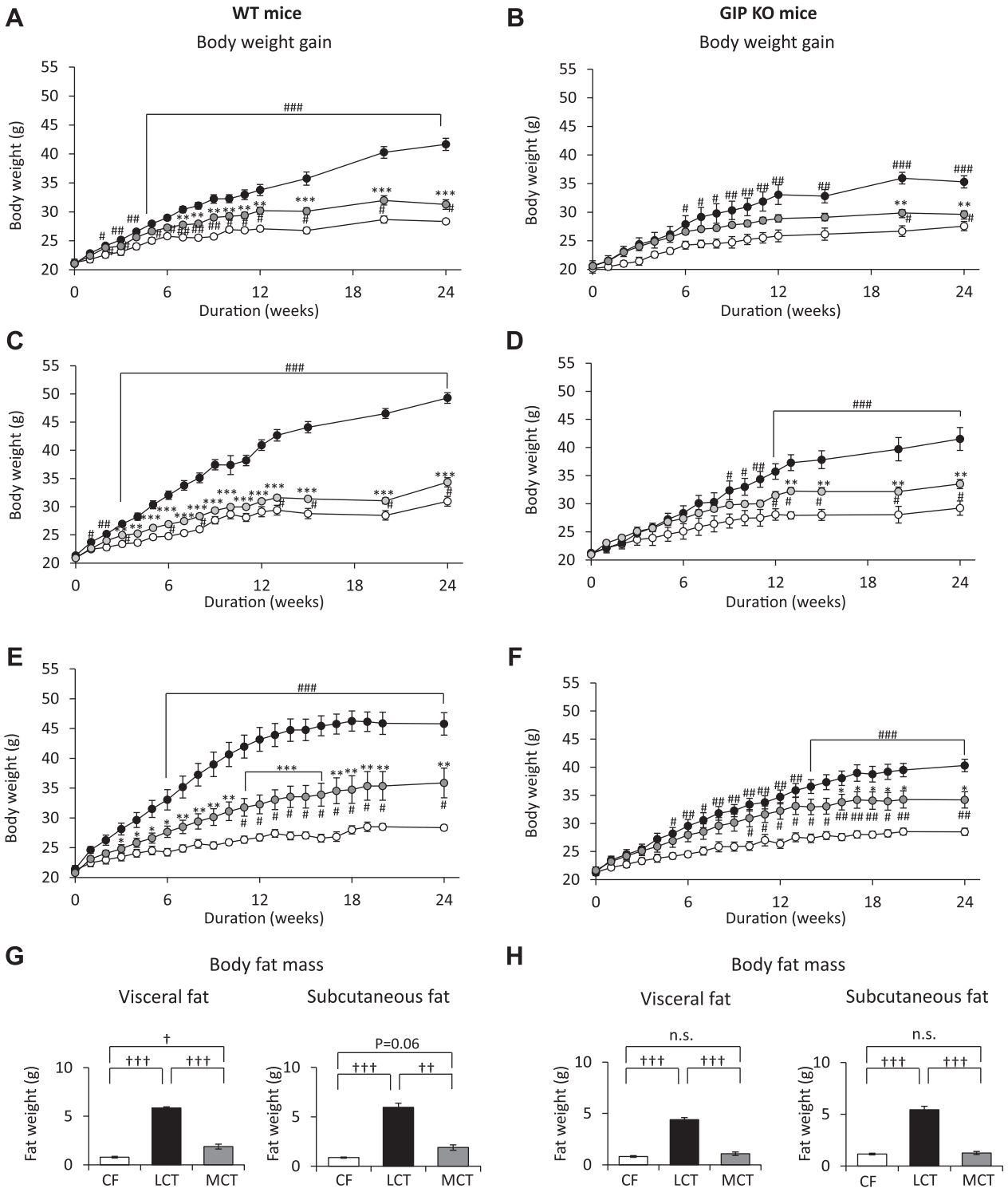

Fig. 2. Body weight gain and body fat mass during diet feeding. Body weight gain in *cohort 1* (*A* and *B*), *cohort 2* (*C* and *D*), and *cohort 3* (*E* and *F*) and body fat mass (*cohort 1*) at 24 wk of diet feeding (*G* and *H*) after long-term diet feeding in wild-type (WT; *A*, *C*, *E*, and *G*) and gastric inhibitory polypeptide (GIP) knockout (KO; *B*, *D*, *F*, and *H*) mice (*cohort 1* and *cohort 3*, $n = 6$ and *cohort 2*, $n = 7$). Control fat (CF) diet-fed mice are represented by white circles and bars. Long-chain triglyceride (LCT) diet-fed mice are represented by black circles and bars. Medium-chain triglyceride (MCT) diet-fed mice are represented by gray circles and bars. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. CF diet-fed mice (one-way ANOVA with Tukey's test). $^{**}P < 0.01$, $^{***}P < 0.001$ vs. LCT diet-fed mice (one-way ANOVA with Tukey's test). †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ (one-way ANOVA with Tukey's test); n.s. no significance.

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61.4% higher than that of the CF diet-fed group at 24-wk diet feeding in WT mice, respectively (Fig. 2, *C* and *E*). On the other hand, the body weight of the MCT diet-fed group was 10.9 and 26.4% higher than that of the CF diet-fed group, respectively. In GIP KO mice, body weight of the LCT diet-fed group was 41.9 and 38.5% higher than that of the CF diet-fed group after 24-wk diet administration, respectively (Fig. 2, *D* and *F*). The body weight of the MCT diet-fed group was 14.6 and 20.0% higher than that of the CF diet-fed group, respectively.

In the LCT diet-fed group, visceral and subcutaneous fat mass was significantly increased by 7.5- and 6.8-fold, respectively, compared with the CF diet-fed group in WT mice, which is consistent with the body weight gain (Fig. 2*G*). In the MCT diet-fed group, visceral fat mass was significantly increased by 2.4-fold and subcutaneous fat mass was increased by 2.2-fold compared with those in the CF diet-fed group, respectively (Fig. 2*G*). In GIP KO mice, visceral and subcutaneous fat mass in the LCT diet-fed group was increased by 5.5- and 4.7-fold, respectively, compared with that in the CF diet-fed group (Fig. 2*H*). Visceral and subcutaneous fat mass in the MCT diet-fed group was comparable with the CF diet-fed group in GIP KO mice.

Effect of MCT diet on food intake, energy expenditure, and locomotor activity in WT and GIP KO mice. Food intake did not differ among the three diet-fed groups in both WT and GIP KO mice (Fig. 3, *A* and *B*). Energy intake of the MCT diet-fed group was significantly increased and that of the LCT diet-fed group tended to be increased compared with that of the CF diet-fed group in WT mice (Fig. 3*A*). However, energy intake

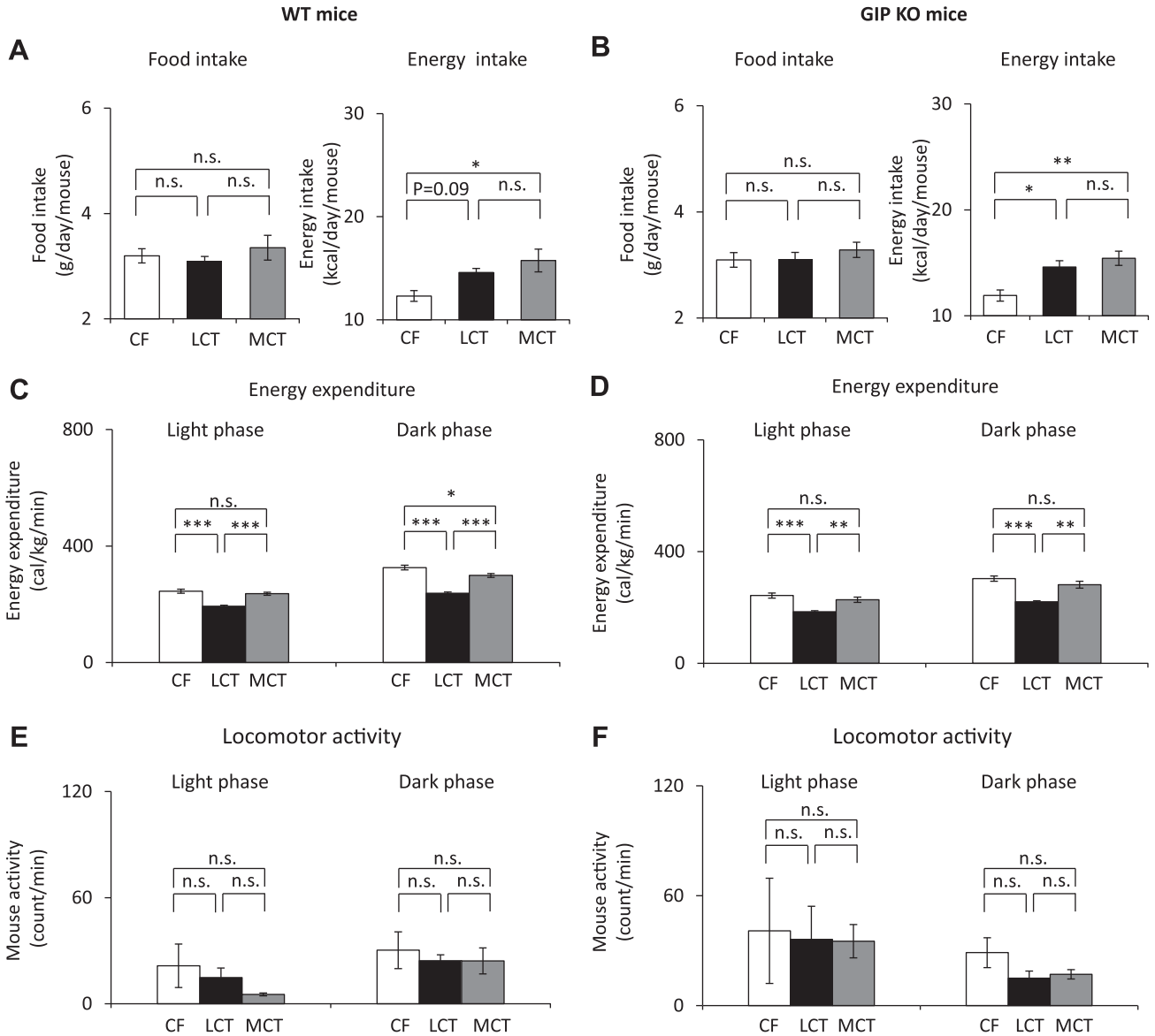

Fig. 3. Food intake, energy expenditure, and locomotor activity during diet feeding. Food intake and energy intake (*A* and *B*) during 2 wk, energy expenditure (*C* and *D*), and locomotor activity at 24–26 wk of diet feeding (*E* and *F*) in wild-type (WT; *A*, *C*, and *E*) and gastric inhibitory polypeptide (GIP) knockout (KO; *B*, *D*, and *F*) mice ($n = 5$). Control fat (CF) diet-fed mice are represented by white bars. Long-chain triglyceride (LCT) diet-fed mice are represented by black bars. Medium-chain triglyceride (MCT) diet-fed mice are represented by gray bars. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (one-way ANOVA with Tukey's test); n.s. no significance.

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did not differ between LCT diet and MCT diet-fed WT mice. In GIP KO mice, energy intake was significantly increased in both the LCT diet- and MCT diet-fed groups compared with that in CF diet-fed group (Fig. 3*B*). However, there was no significant difference in energy intake between LCT diet- and MCT diet-fed GIP KO mice. Energy expenditure of the LCT diet-fed group was significantly lower than that of the CF diet-fed group in both the light and dark phase in WT mice (Fig. 3*C*). In contrast, energy expenditure of the MCT diet-fed group was significantly lower than that of the CF diet-fed group in the dark phase in WT mice. Similarly, energy expenditure of the LCT diet-fed group was significantly lower than that of the CF diet-fed group in GIP KO mice (Fig. 3*D*). However, there was no significant difference in energy expenditure between the MCT diet- and CF diet-fed groups in GIP KO mice. Locomotor activity did not differ among the three diet-fed groups in WT and GIP KO mice (Fig. 3, *E* and *F*).

Total GIP, glucose, and insulin levels during long-term MCT diet feeding. Nonfasting plasma total GIP levels in the LCT diet-fed group were significantly increased compared with those in the CF diet-fed group from the third week of diet administration in WT mice (Fig. 4*A*). In contrast, the total GIP levels in the MCT diet-fed WT mice were similar to those in the CF diet-fed WT mice. In GIP KO mice, GIP levels were under the detectable range (Fig. 4*B*). At the 12th week of diet administration, there was no significant difference in nonfast-

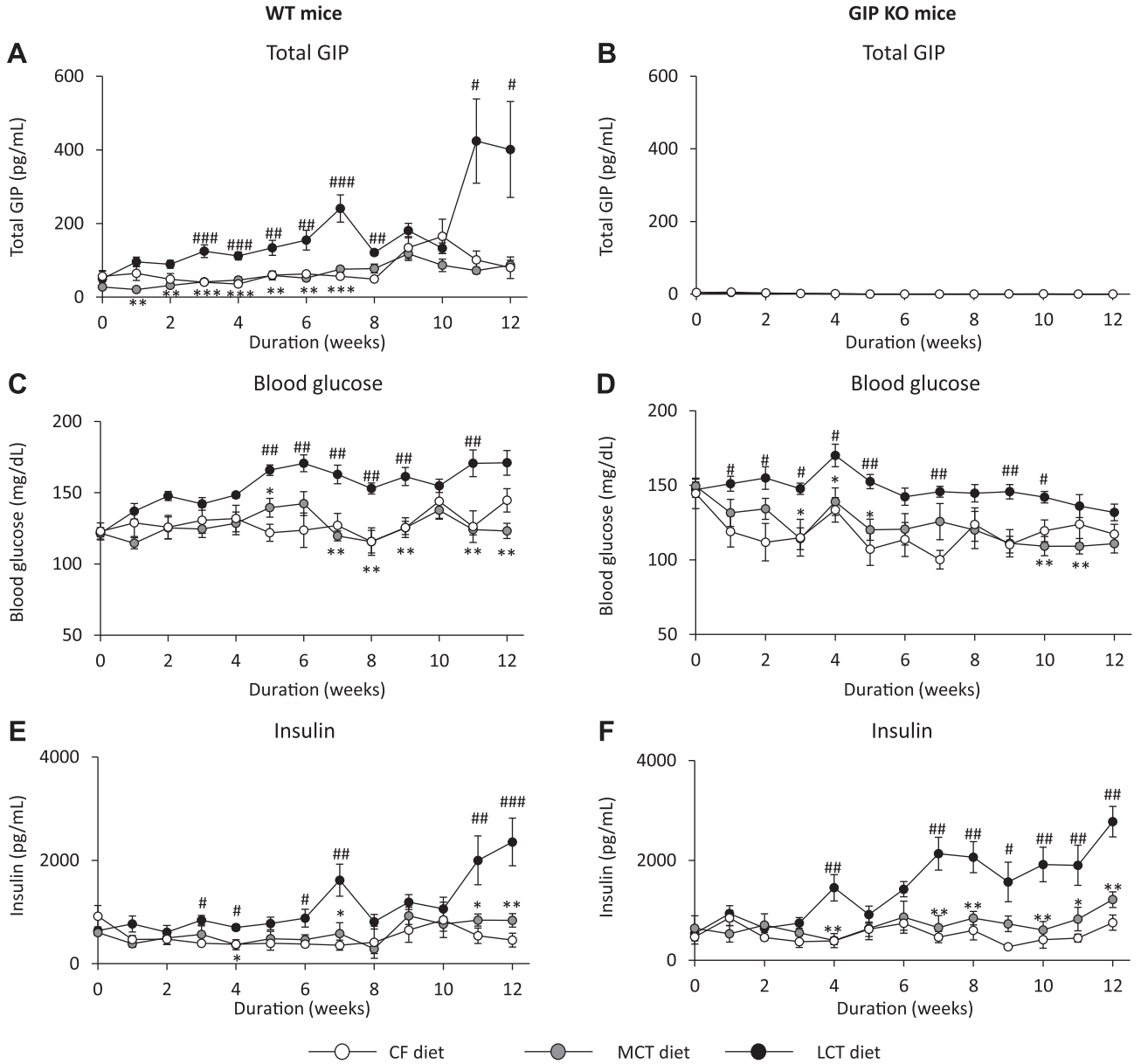

Fig. 4. Nonfasting glucose, total GIP, and insulin levels during diet feeding. Plasma total gastric inhibitory polypeptide (GIP) (*A* and *B*), blood glucose (*C* and *D*), and plasma insulin (*E* and *F*) levels in wild-type (WT; *A*, *C*, and *E*) and gastric inhibitory polypeptide (GIP) knockout (KO; *B*, *D*, and *F*) mice ($n = 6$). Control fat (CF) diet-fed mice are represented by white circles. long-chain triglyceride (LCT) diet-fed mice are represented by black circles. Medium-chain triglyceride (MCT) diet-fed mice are represented by gray circles. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. CF diet-fed mice (one-way ANOVA with Tukey's test). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. LCT diet-fed mice (one-way ANOVA with Tukey's test).

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ing GLP-1 levels among three diet-fed groups in WT and GIP KO mice (data not shown). Nonfasting blood glucose levels were increased in the LCT diet-fed group compared with those in the CF diet-fed group in WT mice from the fifth week of diet administration (Fig. 4*C*). Blood glucose levels in the MCT diet-fed group were comparable to those in CF diet-fed group but were lower compared with those in the LCT diet-fed group. Similarly, in GIP KO mice, nonfasting blood glucose levels in the LCT diet-fed group were higher than those in the CF diet- and MCT diet-fed groups after the second week of diet administration (Fig. 4*D*). Blood glucose levels of the CF diet- and MCT diet-fed groups remained comparable. Plasma insulin levels were significantly higher from the third week of diet administration in the LCT diet-fed group compared with those in the CF diet-fed group but did not differ between the MCT diet- and CF diet-fed groups in WT mice (Fig. 4*E*). In GIP KO mice, insulin levels in the LCT diet-fed group were increased from the seventh week of diet administration compared with those in the CF diet-fed group (Fig. 4*F*). However, there was no significant difference in insulin levels between the MCT diet- and CF diet-fed groups in GIP KO mice.

Effect of MCT diet on glucose tolerance and insulin sensitivity in WT and GIP KO mice. OGTTs were performed in WT and GIP KO mice to evaluate glucose tolerance; blood glucose and plasma insulin levels were measured during OGTT. Blood glucose levels at 15 min were significantly higher in the LCT diet-fed group than those in the CF diet- and MCT diet-fed groups, but AUC blood glucose did not differ among three groups in WT mice (Fig. 5*A*). In GIP KO mice, glucose levels and AUC blood glucose were significantly higher in the LCT diet-fed group than those in the CF diet- and MCT diet-fed groups (Fig. 5*B*). Glucose levels were slightly higher in the MCT diet-fed group than those in the CF diet-fed group, but there was no significant difference between the two groups in GIP KO mice. The LCT diet- and MCT diet-fed groups showed a significant increase in insulin levels compared with the CF diet-fed group, but AUC insulin tended to be lower in the MCT diet-fed group compared with that in the LCT diet-fed group in WT mice (Fig. 5*C*). In GIP KO mice, insulin levels of the LCT diet-fed group were significantly higher compared with those of the CF diet- and MCT diet-fed groups (Fig. 5*D*). In contrast, insulin levels and AUC insulin were similar between the CF diet- and MCT diet-fed groups in GIP KO mice.

Subsequently, ITTs were performed to evaluate insulin sensitivity (Fig. 5, *E* and *F*). The LCT diet-fed group showed higher blood glucose levels after intraperitoneal insulin administration compared with the CF diet- and MCT diet-fed groups in WT mice (Fig. 5*E*). In contrast, the decrease in blood glucose levels was similar between the CF diet- and MCT diet-fed groups in WT mice. In GIP KO mice, blood glucose levels were higher in the LCT diet-fed group than those in the CF diet- and MCT diet-fed groups (Fig. 5*F*). There was no significant difference in blood glucose levels between the CF diet- and MCT diet-fed groups in GIP KO mice.

mRNA expression of molecular markers of obesity, inflammation, lipogenesis, and lipid metabolism in white adipose tissue. Messenger RNA (mRNA) expression levels of obesity markers (adiponectin and leptin), inflammatory cytokines [TNF-α, monocyte chemotactic protein-1 MCP-1, IL-6, and IL-1β], lipogenesis markers [carbohydrate-responsive element-binding protein (ChREBP) and sterol regulatory element-binding protein-1c (SREBP-1c)], and lipid metabolism marker (HSL) were evaluated in white adipose tissue of WT and GIP KO mice.

Expression levels of adiponectin mRNA in the LCT diet-fed group were significantly lower than those in the CF diet- and MCT diet-fed groups in both WT and GIP KO mice (Fig. 6*A*). There was no difference in the expression levels between the CF diet- and MCT diet-fed groups in both WT and GIP KO mice. The expression levels of adiponectin mRNA were higher in GIP KO mice than those in WT mice under the LCT diet-fed condition. Expression levels of leptin mRNA in LCT diet fed group were significantly higher than those in the CF diet- and MCT diet-fed groups in both WT and GIP KO mice (Fig. 6*B*). There was no difference in the expression levels between the CF diet- and MCT diet-fed groups in both WT and GIP KO mice. The expression levels of leptin mRNA tended to be lower in the LCT diet-fed GIP KO mice than those in WT mice but without statistical significance.

The expression levels of TNF-α and MCP-1 mRNA were significantly higher in LCT diet-fed group than those in the CF diet- and MCT diet-fed groups in both WT and GIP KO mice (Fig. 6, *C* and *D*). There was no significant difference in these expression levels between the CF diet- and MCT diet-fed groups in both WT and GIP KO mice. The mRNA expression levels of TNF-α but not MCP-1 were significantly lower in GIP KO mice than those in WT mice under LCT diet-fed condition. The expression levels of IL-6 and IL-1β mRNA were not significantly different among the three diet-fed groups in both WT and GIP KO mice (Fig. 6, *E* and *F*).

The expression levels of ChREBP mRNA in the LCT diet- and MCT diet-fed groups were significantly lower than those in the CF diet-fed group in both WT and GIP KO mice (Fig. 6*G*). The expression levels were not different between the LCT diet- and MCT diet-fed groups in both WT and GIP KO mice. The expression levels of ChREBP mRNA were higher in GIP KO mice than those in WT mice under the LCT diet-fed condition. The expression levels of SREBP-1c mRNA in the MCT diet-fed group were significantly lower than those in the CF diet-fed group but higher than those in the LCT diet-fed group in both WT and GIP KO mice (Fig. 6*H*).

The expression levels of HSL mRNA in the LCT diet-fed group were significantly lower than those in the CF diet- and MCT diet-fed groups in both WT and GIP KO mice (Fig. 6*I*). There was no difference in the expression levels between the CF diet- and MCT diet-fed groups in both WT and GIP KO mice. The expression levels of HSL mRNA were significantly higher in GIP KO mice than those in WT mice under the LCT diet-fed condition.

DISCUSSION

We previously reported that single fat administration induces GIP hypersecretion (56) through fatty acid-binding protein 5 (FABP5) (42) and free fatty acid receptor 1 (GPR40) (39) and 4 (GPR120) (20). In addition, long-term HFD intake causes GIP hypersecretion (30), most likely due to increased GIP gene expression (5) via transcriptional factor regulatory factor X6 (Rfx6) and pancreatic and duodenal homeobox 1 (Pdx1) (13, 50). Since both GIPR KO mice (26) and GIP KO mice (29) showed resistance to HFD-induced obesity and

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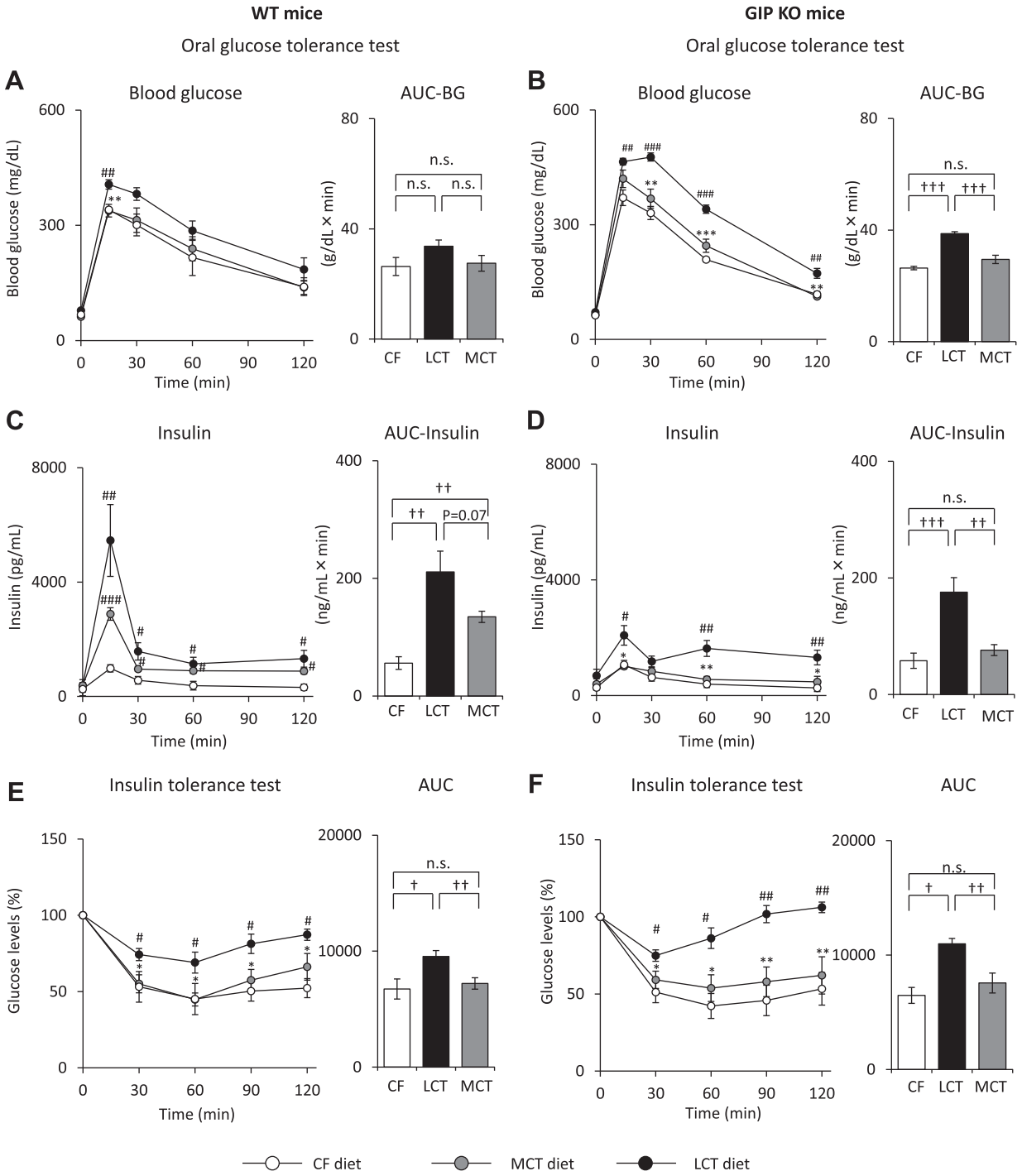

Fig. 5. Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) after diet feeding. Plasma blood glucose (BG; *A* and *B*) and plasma insulin (*C* and *D*) levels during OGTT at 13 wk of diet feeding in wild-type (WT; *A* and *C*) and gastric inhibitory polypeptide (GIP) knockout (KO; *B* and *D*) mice ($n = 5$). Blood glucose levels during ITT at 20 wk of diet feeding in WT (*E*) and GIP KO (*F*) mice. The glucose levels during ITT represent the percentage change from fasting blood glucose levels. Control fat (CF) diet-fed mice are represented by white circles and bars. Long-chain triglyceride (LCT) diet-fed mice are represented by black circles and bars. Medium-chain triglyceride (MCT) diet-fed mice are represented by gray circles and bars. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. CF diet-fed mice (one-way ANOVA with Tukey's test). AUC, area under the curve. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. LCT diet-fed mice (one-way ANOVA with Tukey's test). $\dagger P < 0.05$, $\dagger\dagger P < 0.01$, $\dagger\dagger\dagger P < 0.001$ (one-way ANOVA with Tukey's test); n.s. no significance.

insulin resistance, HFD-induced GIP hypersecretion was considered to increase the volume of adipose tissue by binding to GIPR in the adipose tissue (23, 24, 45) and by promoting insulin secretion from pancreatic β-cells under HFD feeding (34). However, these studies were performed using HFD based on LCT. Therefore, we investigated the effect of single MCT oil administration on incretin secretion and also compared the effects of long-term LCT diet and MCT diet intake on incretin secretion, obesity, and insulin resistance.

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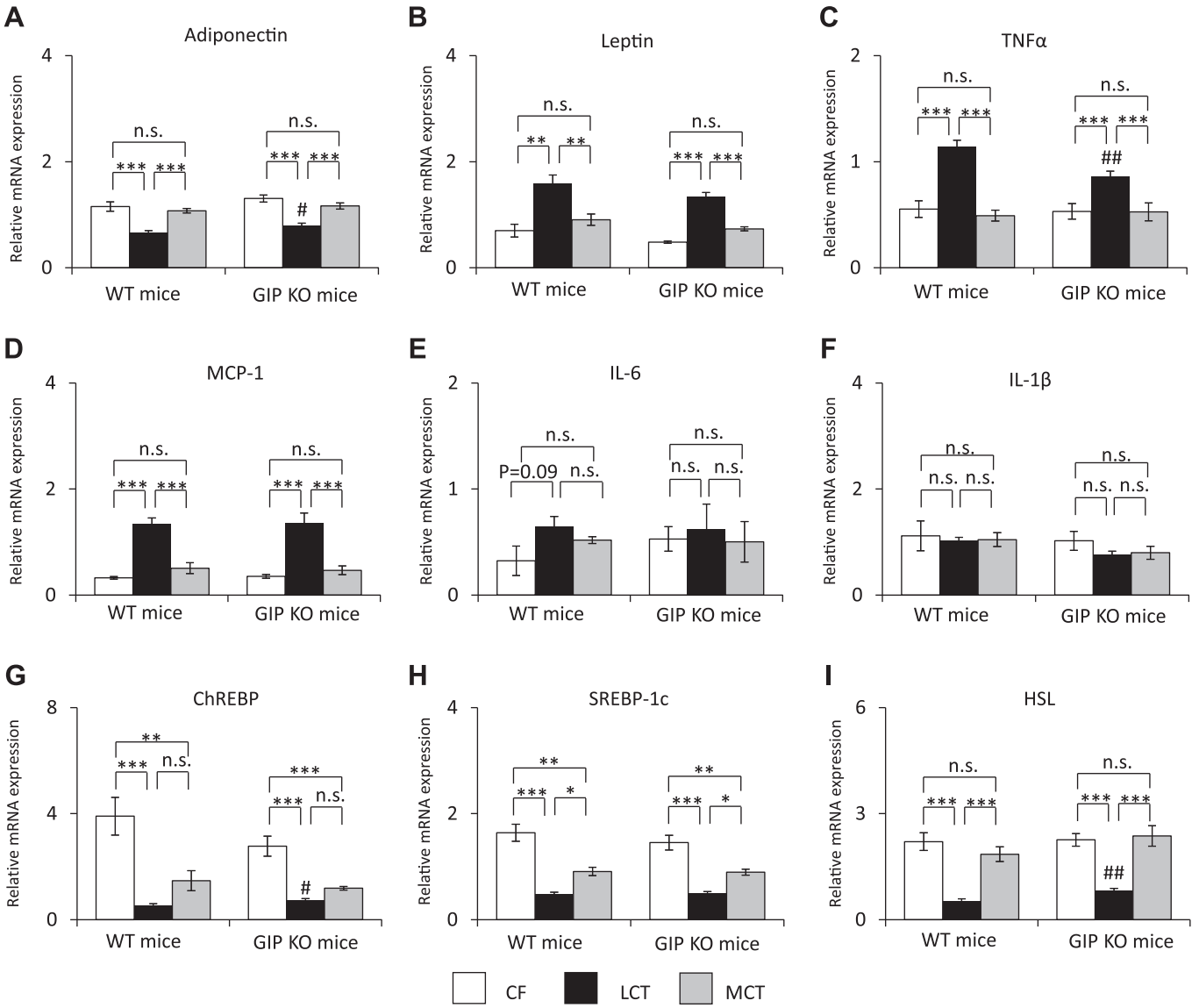

Fig. 6. Relative expression levels of markers of obesity, inflammation, and lipogenesis and lipid metabolism. Expression levels of adiponectin (*A*), leptin (*B*), TNF-α (*C*), monocyte chemotactic protein-1 (MCP-1; *D*), IL-6 (*E*), and IL-1β (*F*), carbohydrate-responsive element-binding protein (ChREBP; *G*), sterol regulatory element-binding protein-1c (SREBP-1c: *H*), and hormone-sensitive lipase (HSL; *I*) mRNA in visceral fat at 24 wk of diet feeding in WT and GIP KO mice ($n = 6$). Control fat (CF) diet-fed mice are represented by white bars. Long-chain triglyceride (LCT) diet-fed mice are represented by black bars. Medium-chain triglyceride (MCT) diet-fed mice are represented by gray bars. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (one-way ANOVA with Tukey's test). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. WT mice (Student *t*-test); n.s. no significance.

MCT diet has a beneficial role in reduction of body weight and body fat mass compared with LCT diet. Some studies have revealed mechanisms of suppression of body weight and fat mass by MCT diet such as activation of fatty acid oxidation in the liver, increased energy expenditure, and changes in the intestinal bacterial flora (44, 52, 57). In this study, single administration of LCT oil induced both GLP-1 and GIP secretion, whereas single administration of MCT oil stimulated GLP-1 secretion but not GIP secretion in lean mice. Thus we hypothesized that MCT diet induces less body weight and fat mass gain compared with LCT diet due to stunted GIP secretion. In the present study, long-term (24 wk) LCT- or MCT-based HFD-feeding experiments revealed that LCT diet induces GIP hypersecretion and more body weight and fat mass gain compared with CF diet, whereas MCT diet stimulates less GIP secretion and suppresses body weight and fat mass gain compared with LCT diet despite the same energy density. The long-term LCT diet-fed group showed GIP hypersecretion and a 50–60% increase in body weight compared with the CF diet-fed group in WT mice. In contrast, long-term MCT diet did not induce GIP hypersecretion, and the increase in body weight in the MCT diet-fed group was only 10–25% compared with that in the CF diet-fed group in WT mice. In GIP KO mice, body weight gain of the LCT diet-fed group was not comparable to that of the CF diet-fed group, but the increase in body weight of LCT diet-fed GIP KO mice (25–40% increase compared with CF diet-fed mice) was smaller than that of LCT diet-fed WT mice (50–60%). In contrast, there was a marginal difference in body weight gain between MCT diet-fed GIP KO (10–20%) and MCT diet-fed WT mice (10–25%). Taken

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together, the difference in body weight gain between LCT diet and MCT diet still remains but is much smaller in GIP KO mice compared with that in WT mice. These results suggested that GIP is not only one contributor to the phenotype of LCT diet versus MCT diet but plays an important role for the difference in body weight and fat mass between the two diet groups. Adipose tissue plays a critical role in energy storage (37). GIP increases glucose and LCT uptake in adipose tissue and enhances energy storage into adipose tissue (55). LCTs absorbed from the intestine are transported to adipose tissue via the lymphatic system and are mainly stored in adipose tissue (2, 3). In contrast, MCFAs digested from MCTs in the intestinal tract are transported to the liver via the portal vein and are β-oxidized for production of energy in te liver. Thus the difference in contribution of GIP to body weight and fat mass between LCT diet and MCT diet might be due to not only the amount of GIP secretion but also to different utilization of triglycerides in vivo.

Long-term HFD intake based on LCT induces insulin resistance as well as obesity (49). Excessive fat storage decreases the expression levels of insulin sensitivity-related hormones and molecules such as adiponectin and HSL (6, 26) and induces leptin resistance and the production of inflammatory cytokines such as TNF-α, MCP-1, IL-6, and IL-1β (54). Consistent with these observations, insulin sensitivity and expression levels of adiponectin and HSL mRNA in adipose tissue were decreased in LCT diet-fed WT mice compared with those in CF diet-fed WT mice. In addition, expression levels of leptin, TNF-α, and MCP-1 mRNA were increased more in LCT diet-fed WT mice than in CF diet-fed WT mice. On the other hand, long-term MCT diet feeding is reported to improve insulin sensitivity in rodents and humans (15, 16). In this study, MCT diet-fed WT mice showed similar insulin sensitivity and expression levels of adiponectin, leptin, HSL, TNF-α, and MCP-1 mRNA in adipose tissue to CF diet-fed WT mice. This is possibly due to the difference in body weight and fat mass gain between LCT diet- and MCT diet-fed mice. Thus long-term MCT diet does not induce insulin and leptin resistance and inflammation due to alleviation of excessive fat storage.

Earlier studies have indicated that GIP plays a critical role in the compensatory enhancement of insulin secretion under obesity and insulin resistance (17, 31). In the present study, LCT diet-fed WT mice showed compensatory insulin secretion to preserve blood glucose levels during OGTT. On the other hand, insulin secretion was impaired in LCT diet-fed GIP KO mice, resulting in hyperglycemia after glucose ingestion. These results strongly indicate that GIP has a critical role in compensatory insulin secretion for maintenance of blood glucose levels during OGTT in LCT diet-fed mice. MCT diet-fed WT mice showed similar glucose levels to CF diet-fed WT mice by increasing insulin secretion after glucose ingestion. In GIP KO mice, postprandial hyperglycemia was not observed in the MCT diet-fed group, although insulin levels did not differ between the CF diet- and MCT diet-fed groups. Therefore, GIP contributes to the preservation of postprandial glucose levels under the LCT diet-fed condition, whereas the effect of GIP is smaller under the MCT diet-fed condition.

The strength and novelty of our study design are the simultaneous comparison of the effects of MCT diet and LCT diet in both WT and GIP KO mice. To the best of our knowledge, this is the first study to demonstrate the contribution of GIP to body weight, fat mass, and glucose tolerance under long-term MCT diet feeding. From the results of our present study, the clinical use of MCT diet might enable suppression of GIP secretion and thereby reduce the risk of obesity.

In conclusion, MCT diet stimulated less GIP secretion and suppressed body weight and fat gain compared with LCT diet, resulting in alleviation of adiposity. The composition of dietary fat is a key determinant of GIP secretion and therefore might affect body weight and body fat mass.

ACKNOWLEDGMENTS

We thank Shoichi Asano (Department of Diabetes, Endocrinology and Nutrition, Graduate School of Medicine, Kyoto University) for technical support regarding the study.

GRANTS

This study was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology; Japan Society for the Promotion of Science; Ministry of Health, Labour, and Welfare; Ministry of Agriculture, Forestry and Fisheries; Japan Diabetes Foundation; Japan Association for Diabetes Education and Care; Merck Sharp & Dohme Life Science Foundation; Public Interest Incorporated Foundation; Japan Diabetes Foundation; and Suzuken Memorial Foundation.

DISCLOSURES

N. Inagaki receives fees from Takeda Pharmaceutical, Mitsubishi Tanabe Pharma, Merck Sharp & Dohme, Sanofi, Novartis Pharma, Dainippon Sumitomo Pharma, Kyowa Hakko Kirin, Eli Lily Japan, Roche Diagnostics, Japan Tobacco, Nippon Boehringer Ingelheim, Astellas Pharma, Daiichi Sankyo, Ono Pharmaceutical, and Taisho Toyama Pharmaceutical. N. Harada receives fees from Novo Nordisk Pharma, Mitsubishi Tanabe Pharma, and Ono Pharmaceutical. Y. Murata, S. Yamane, K. Iwasaki, E. Ikeguchi, Y. Kanemaru, T. Harada, A. Sankoda, S. Shimazu-Kuwahara, E. Joo, and H. Poudyal have no conflict of interest to disclose.

AUTHOR CONTRIBUTIONS

Y.M., N.H., S.Y., K.I., and N.I. conceived and designed research; Y.M. performed experiments; Y.M. analyzed data; Y.M., N.H., S.Y., K.I., E.I., Y.K., T.H., A.S., S.S.-K., E.J., H.P., and N.I. interpreted results of experiments; Y.M. prepared figures; Y.M. drafted manuscript; Y.M., N.H., E.I., H.P., and N.I. edited and revised manuscript; Y.M., N.H., S.Y., K.I., E.I., Y.K., T.H., A.S., S.S.-K., E.J., H.P., and N.I. approved final version of manuscript.

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