

ORIGINAL ARTICLE

Gastric inhibitory polypeptide receptor antagonist, SKL-14959, suppressed body weight gain on diet-induced obesity mice

T. Nakamura¹ , H. Tanimoto¹, Y. Mizuno², M. Okamoto¹, M. Takeuchi¹, Y. Tsubamoto¹ and H. Noda¹

¹Biological Research Group, Drug Discovery Laboratories, Sanwa Kagaku Kenkyusho, Inabe-city, Mie, Japan; ²Biopharmaceutical Study Group, Pharmaceutical Research Laboratories, Sanwa Kagaku Kenkyusho, Inabe-city, Mie, Japan;

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Correspondence:

Takashi Nakamura, Biological Research Group, Drug Discovery Laboratories, Sanwa Kagaku Kenkyusho Co., LTD., 363 Shiosaki, Hokusei-Cho, Inabe-City, Mie 511-0406, Japan.
E-mail: ta_nakamura@mb4.skk-net.com

Summary**Objective**

Gastric inhibitory polypeptide plays a role in glucose and lipid metabolism and is associated with obesity and insulin resistance. The objective of this study is to confirm the anti-obesity effects of the gastric inhibitory polypeptide receptor antagonist, SKL-14959, on diet-induced obesity mice.

Method

Diet-induced obesity mice at 20 weeks of age were administered with or without SKL-14959 for 96 d. Body weight and food intake were monitored throughout the experiment. Mice were sacrificed, and physiological and biochemical markers were measured, and then histochemical and gene expression analyses were also performed. In further studies, mice were orally gavaged with [¹⁴C]-oleic acid to investigate the excursion of digested lipids.

Results

SKL-14959 significantly suppressed weight gain without affecting food intake, decreased triacylglycerol contents in the liver and the muscle and the intensity stained with oil-red in the liver. It also improved plasma glutamic pyruvic transaminase and 3-hydroxybutyrate levels in addition to notably down-regulated relative gene expression of *srebf1* and *dgat1* in the liver despite not altering in the adipose tissue. Furthermore, SKL-14959 showed remarkable inhibition of lipid uptake in the adipose tissue after the oil challenge.

Conclusion

SKL-14959 inhibited lipids uptake and improved lipids metabolism, results in suppression of body-weight gain.

Keywords: anti-obesity, fatty liver, GIP, GIP receptor antagonist.

Introduction

Obesity has been a major health problem in developed countries and is a growing issue in the developing world. The world-wide epidemic of obesity is about to increase the risk of the incidence of diabetes mellitus, insulin resistance, fatty liver, hyperlipidaemia, hypertension, cardiovascular diseases and some forms of cancer (1,2). Several anti-obesity agents such as orlistat, sibutramine and rimonabant have been approved for clinical uses in many countries including in the United States or Europe. However, these drugs with the exception of orlistat were

withdrawn from the market due to the serious adverse effects of cardiac dysfunction or psychiatric behaviour (3). Recently, lorcaserin and exendine, a selective serotonin 2C (5HT_{2c}) receptor agonist and Qsymia, a combination of phentermine and topiramate, and liraglutide were approved by the US Food and Drug Administration (4). Thus, an ideal anti-obesity agent without adverse effects has yet to be developed.

Gastric inhibitory polypeptide or glucose-dependent insulinotropic polypeptide (GIP) is an incretin hormone, which is released from duodenal K-cell in a glucose-dependent manner (5,6). The physiological function of

GIP plays a key role in promoting fatty acid incorporation and synthesis (7,8) and enhancing of lipoprotein lipase (LPL) activity in explant adipose tissues or 3 T3-L1 cell (9,10). Increased postprandial GIP levels have been associated with obesity in both human (11–15) and mice (16). Furthermore, GIP infusion increased the rate of chylomicron clearance in dogs (17), and both endogenous and exogenous GIP were shown to lower plasma triacylglycerol (TG) levels in rats in response to intraduodenal fat infusion (18).

Gastric inhibitory polypeptide receptor knock-out mice fed high-fat diets showed suppression of body weight gain compared with wild mice fed the same high-fat diets (19). In addition, Nasteska et al. (20) reported that chronic reduction of GIP improved obesity and insulin resistance in transgenic mice fed a high-fat diet. Moreover, administration of GIP receptor antagonist, (Pro3) GIP, prevented the development of obesity and insulin resistance in diet-induced obesity (DIO) mice and *ob/ob* mice (21,22). However, this compound has also the effect of a partial or full agonist against GIP receptor (23). Nakamura et al. (24) reported that a candidate, SKL-14959 is a novel low molecular chemical compound and GIP specific receptor antagonist, had a strong binding affinity for the GIP receptor and inhibited GIP-stimulated cAMP production. Additionally, SKL-14959 suppressed insulin secretion in oral glucose tolerance test and postponed TG clearance from the circulation in oil tolerance test. In the point of clinical applications, Roux-en-Y gastric bypass surgery, which includes the removal of GIP-secreting K cells from the upper small intestine, results in body weight loss and improved insulin resistance (25). Thus, it seems that GIP has a bifacial characterization, at least, it is apparent from the contribution to obesity, insulin resistance and diabetes mellitus.

The objective of the present study is to confirm the hypothesis that inhibition of lipid uptake by GIP receptor antagonist, SKL-14959 on DIO mice model, can indicate an anti-obesity effect.

Material and methods

SKL-14959 was synthesized in our laboratory. [^{14}C]-oleic acid was purchased from Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan). All other chemicals used were of molecular biology grade.

Animal study

Diets-induced obesity model

All experiments were approved by the Committee on Animal Care of Sanwa Kagaku Kenkyusho, SKK. Institute of

Cancer Research (ICR) male mice obtained from Clea Japan (Osaka, Japan) at 10 weeks of age were fed laboratory standard powder chow, CRF-1 (Oriental Yeast Co., Ltd., Tokyo, Japan). Mice were allowed *ad libitum* access to food and water and were maintained in a plastic cage at a constant temperature of $23 \pm 2^\circ\text{C}$ under a 12-h light–dark cycle (lights on from 7:00 am to 7:00 pm). At 16 weeks of age, mice were divided into two groups of low-fat diet (LFD) fed (Oriental Yeast Co., Ltd. Tokyo, Japan; fat 5%, protein 16.8%, carbohydrate 60.9%; $358.9 \text{ kcal } 100 \text{ g}^{-1}$) and high-fat diet (HFD) fed (Oriental Yeast Co., Ltd., Tokyo, Japan; beef tallow 40%, corn starch 10%, sugar 9%, vitamin mixture 1%, mineral mixture 4% and casein 36%; $563.4 \text{ kcal } 100 \text{ g}^{-1}$) (26) and acclimated individually in plastic cages.

Drug evaluation

At 20 weeks of age, the HFD group was divided into two groups, HFD control and treatment with SKL-14959, the body mass and food intake were matched, and the study was performed to evaluate anti-obesity for 95–96 d. The beginning of administration was defined as day1. The treatment group was given SKL-14959 by 0.133% mixed diets during the test. Body mass and food intake were measured until the end of the study. Anatomy was split into 2 d to match the condition of mealtimes in a day. Mice were anaesthetized using ether and mouse lengths, from nose-to-anus, and were measured using callipers before the mice were sacrificed and dissected. Body mass index (BMI) was calculated as body weight (g) divided by the length (cm) squared. Blood samples were withdrawn from inferior vena cava and centrifuged to separate plasma and then kept at -80°C until each was assayed. Liver, heart, kidney, spleen, lung, adipose tissues, mesenteric, epididymal, perirenal and retroperitoneal adipose pads and soleus, gastrocnemius and vastus lateralis muscles were removed, and their wet weight measured. For histological analyses, sections of the liver and adipose tissue were either immediately stored in liquid nitrogen or fixed in 10% neutral-buffered formalin. The remaining sections of the liver and adipose tissue were immediately transferred to -80°C and stored until TG and gene expression analysis were performed.

Lipid distribution in single [^{14}C]-oleic acid administration

ICR male mice at 20 weeks of age were divided into two groups on the basis of body weight in the morning after 16-h fast and then orally administered with or without SKL-14959 ($100 \text{ mg } \text{kg}^{-1}$ body weight) prepared with

0.5% sodium carboxymethylcellulose solution 15 min prior to oral administration of [^{14}C]-oleic acid (18.5 MBq g^{-1}). The mice were immediately released into an occlusive metabolic cage Metabolica from Yamagen Co., Ltd. (Tokyo, Japan) after the challenge and then euthanized under ether anaesthesia at 60 and 120 min. Blood samples were collected from the inferior vena cava, and epididymal adipose tissue, soleus muscles and liver were removed and weighed. Sections of all samples including blood were oxidized using a combustion system (ASC-113, Aloka), and the radioactivity in the absorbent (Carbo-Sorb E, PerkinElmer), which absorbed yields of ^{14}C -carbon dioxide, was measured using a liquid scintillation counter (LS6000TA, Beckman). Those data were expressed as a concentration of each whole tissue.

Triacylglycerol content

Triglyceride analysis was measured with the modified procedure previously reported (27). In brief, the liver and the muscle samples were homogenized in chloroform-methanol mixture (2:1 v/v) and then extracted with the same reagent and phosphate buffer saline. Organic layers were dried up under the stream of nitrogen and reconstructed in isopropanol-TritonX-100 (9:1). The TG content was determined using L-type Wako TG-H (Wako Pure Chemicals, Osaka, Japan).

Histological analysis

The small piece of the epididymal adipose tissue fixed with 10% neutral-buffered formalin was embedded in paraffin. These paraffin blocks were sectioned at a thickness of 2 μm and stained with eosin and haematoxylin. To examine the size of adipocytes, the number of cells was individually counted three areas scanned under the 15 power 20 magnification and then expressed as the mean \pm standard error (i.e. a larger number means smaller size) (28). The small piece of the liver fixed with 10% neutral-buffered formalin was dipped into 10% to 20% sucrose solution in 0.1 M phosphate buffer at 4°C all day and then frozen in liquid nitrogen with freezing medium (compound Tissue-Tek). From the blocks, 10 μm -thick sections were prepared and then stained with Oil red O. To examine the degree of steatosis, images were captured using a microscope (Olympus BX-50, Tokyo, Japan) under the 10 power 10 magnification.

β -Hydroxyacyl-CoA dehydrogenase assay

β -Hydroxyacyl-CoA dehydrogenase (β -HAD) assay was measured with the procedure previously reported (29).

Liver and muscle segments were weighed and immediately homogenized in 20 volumes of ice-cold 20 mM phosphate buffer (pH 7.4) including in 5.4 M glycerol, 5 mM 2-mercaptoethanol, 0.5 mM ethylenediaminetetraacetic acid and 0.02% bovine serum albumin. The homogenate was centrifuged 600 g for 10 min before the supernatant was used for enzyme assay. All homogenate operations were performed at 0–4°C. The reaction mixture contained 95 mM pyrophosphate buffer (pH 7.3) including in 0.2 mM nicotinamide adenine dinucleotide, 4.8 mM acetoacetyl-CoA as a substrate and its supernatant. The activity was determined for 5 min at 25°C by monitoring the rate of disappearance of nicotinamide adenine dinucleotide by measuring the absorbance at 339 nm. One unit of the activity was defined as the conversion of 1.0 μmol^{-1} of substrate to product per minute and was expressed as U g^{-1} tissue.

RNA extraction and real-time polymerase chain reaction

Total RNA was isolated from the frozen liver and the adipose tissues using the RNeasy Mini Kit (QIAGEN, Valencia, CA) following the manufacturer's instruction. In brief, approximately 100 mg organs were homogenized in 1 mL of Trizol reagent (Invitrogen, Carlsbad, CA) using zirconia beads and were purified total RNA. Two micrograms of total RNA was reverse-transcribed to complementary DNA using the High Capacity cDNA Reverse Transcription Kit (Applied biosystems, Foster City, CA). Polymerase chain reactions were performed using the LightCycler480 (Roche, USA). Polymerase chain reactions for each target cDNA were carried out using commercially supplied ready-to use primer and probe sets by Applied Biosystems (TaqMan gene expression assays): sterol regulatory element binding transcription factor 1, *Srebf1* (Mm00550338_m1), diacylglycerol O-acyltransferase 1, *Dgat1* (Mm00515643_m1), fatty acid synthase, *Fasn* (Mm01253292_m1), acetyl-coenzyme A carboxylase alpha, *Acaca* (Mm01304257_m1), peroxisome proliferator activated receptor alpha, *Ppara* (Mm00440939_m1), uncoupling protein 2, *Ucp2* (Mm00627598_m1), hormone sensitive lipase, *Lipe* (Mm00495359_m1) and TATA box binding protein, *TBP* (Mm0111222) as housekeeping gene. Relative gene expression was analysed by the $\Delta\Delta\text{CT}$ method (30) and normalized with internal control, TBP. We validated TBP as housekeeping gene by confirming its expression level was not significantly different between each group. LFD was defined as 1.0 and expressed mean \pm standard deviation as fold change.

Blood analysis

Plasma glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), non-esterified free acid (NEFA), TG, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and glucose levels (Glu) were measured with L-type Wako GOT-J2, L-type Wako GPT-J2, NEFA-HA Test Wako, L-type Wako TG-H, L-type CHO H, L-Type LDL-C, L-Type HDL-C and L-Type Glucose 2 (Wako Pure Chemical Industries, Osaka, Japan), respectively. 3-Hydroxybutyrate was determined by Ketone test liquid (Sanwa Kagaku Kenkyusho, Nagoya, Japan) using auto analyser (7170, Hitachi Ltd., Tokyo, Japan). Plasma GIP, insulin, and adiponectin levels were measured using enzyme-linked

immunosorbent assay kits (LINCO RESEARCH, INC., Missouri, U.S.A., Shibayagi, Gumma, Japan, and Otsuka Pharmaceutical Ltd., Tokushima, Japan, respectively).

Statistical analysis

Data were expressed as means ± standard error. Two-way analysis of variance followed by Dunnett's test were performed in the body weight, body weight gain and food intake, and others were performed by unpaired *t*-test for comparison of the LFD and the HFD group or of the HFD treated with or without SKL-14959 group and less than 0.05 of *p* value was statistically significant.

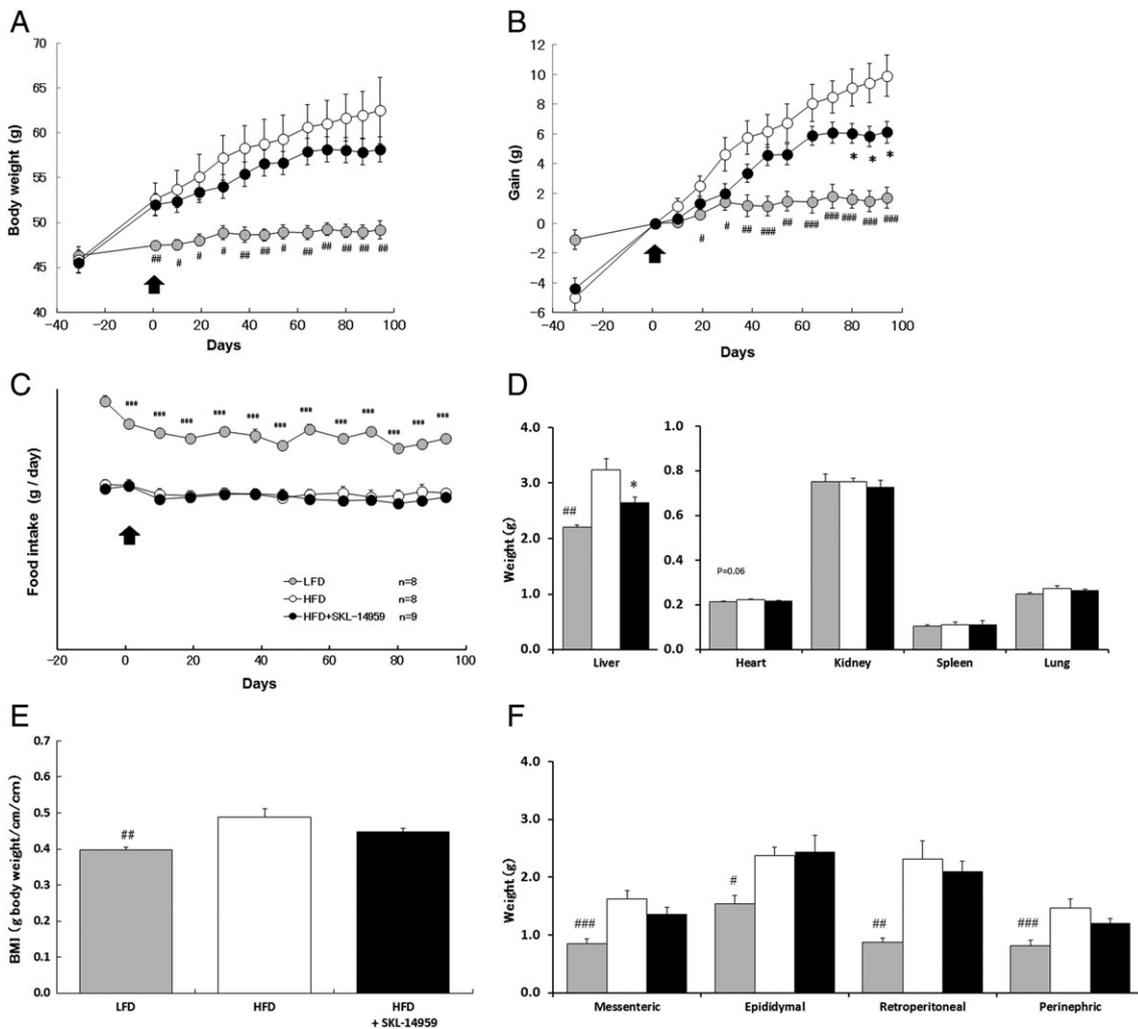


Figure 1 Effects of SKL-14959 on body weight (A), body weight gain (B), daily food intake (C), organ weights (D), body mass index (BMI) (E) and adipose tissue weights (F). Grey, open and closed circle or bar express LFD, HFD and HFD with SKL-14959 group, respectively. Arrow is the date of beginning administration, Day1. Data are means ± standard error (eight to nine mice per group). # *p* < 0.05, ## *p* < 0.01, ### *p* < 0.001 vs. LFD group, **p* < 0.05 vs. HFD control group (*t*-test).

Results

Effects of SKL-14959 treatment on body weight gain and food intake and tissue mass

Two-way repeated measures analysis of variance (mouse groups across time) was used to analyse body weight, weight gain, food intake and tissue mass. Intake calories in the LFD, HFD control and SKL-14959-treated group through the test were 1638 ± 6.6 , 1877 ± 10 and 1845 ± 9.5 kcal, respectively. The body mass gain in the HFD control group was significantly increased compared with the LFD group, whereas the food intake was significantly decreased. The body weight gain in the SKL-14959-treated group was significantly suppressed compared with the HFD control group without the impact of food intake ($p < 0.001$), and the suppression percent of body mass was 7.0% (Figure 1A–1C). BMI was not significantly decreased (Figure 1E).

Weights of the each adipose pad, mesenteric, epididymal, retroperitoneal, and perirenal, the liver and BMI in the HFD control group were significantly increased compared with the LFD group ($p < 0.01$). The SKL-14959-treated group was significantly decreased in the liver weight ($p < 0.05$) and was not significantly decrease BMI and the adipose tissues compared with the HFD control group (Figure 1D and 1F). The heart, kidney, spleen and lung

weight did not show important differences between all groups (Figure 1D).

Biochemical markers in plasma

Plasma GPT activity and total cholesterol, LDL, HDL, GIP and insulin concentrations in the HFD control group were significantly elevated compared with the LFD group, whereas the SKL-14959-treated group tended have lower plasma levels of GOT and GPT activity ($p = 0.08$ and $p < 0.05$, respectively), 3-hydroxybutyrate ($p = 0.09$) compared with the HFD control group. Plasma NEFA, TG, glucose and adiponectin concentrations were not significantly different between all groups (Table 1).

Triacylglycerol content and β -hydroxyacyl-CoA dehydrogenase activity in the liver and the muscles

Hepatic and muscle lipid accumulation in the HFD control group were increased compared with the LFD group. TG contents in the SKL-14959-treated group showed significant decrease in the liver ($p < 0.05$) and showed a tendency to decrease in the muscles contrasted with the HFD control (Figure 2A–2C). Hepatic and muscle β -HAD activities in the HFD control group were increased compared with the LFD group, but there was no significant difference between the results for the SKL-14959-treated group and the HFD control group (Figure 2D–2F).

Histological analysis of the liver and the adipocytes

In histochemical analysis in the liver, the HFD control group showed significant higher intensity by oil-red staining compared with the LFD group ($p < 0.001$), whereas the SKL-14959-treated group showed a significant decrease in the intensity compared with the HFD control group ($p < 0.05$, Figure 3A–3D). In the adipose tissues, the size of the epididymal adipocytes in the HFD control group was significantly larger than the LFD group ($p < 0.001$), but the sizes of the adipocytes were not significantly different between the SKL-14959-treated group and the HFD control group (Figure 3E–3H).

Relative gene expression using real-time polymerase chain reaction

To investigate gene expression levels regulating lipogenesis and lipolysis in the liver and the adipose tissue, we performed gene analysis. In the liver, the HFD control group showed significant enhancement of relative gene expression of SREBP-1 (*Srebp1*) and PPAR-alpha (*Ppara*) compared with the LFD group ($p < 0.05$), whereas the SKL-14959-treated group showed significant down-

Table 1 Plasma parameters treated with SKL-14959 for 95–96 days on diet-induced obesity in mice.

Marker		LFD	HFD	HFD + SKL-14959
GOT	(IU/L)	46 \pm 9.0	60 \pm 6.6	47 \pm 2.9
GPT	(IU/L)	27 \pm 3.2	48 \pm 7.2*	27 \pm 2.8***
NEFA	(μ Eq/L)	1.80 \pm 0.1	1.76 \pm 0.2	1.98 \pm 0.2
TG	(mg/dL)	100 \pm 11	108 \pm 17	122 \pm 19
T-Chol	(mg/dL)	112 \pm 3.9	209 \pm 5.4**	190 \pm 7.8
LDL	(mg/dL)	11.6 \pm 0.9	38 \pm 7.1**	28 \pm 3.2
HDL	(mg/dL)	88.5 \pm 3.4	165 \pm 12**	153 \pm 6.1
Glucose	(mg/dL)	213 \pm 9.7	253 \pm 11	232 \pm 9.3
3-hydroxybutyrate	(mg/dL)	132 \pm 9.7	193 \pm 31	135 \pm 14
GIP	(pg/mL)	28.7 \pm 5.5	75.2 \pm 14.4**	87 \pm 10
Insulin	(ng/mL)	3.4 \pm 1.3	9.0 \pm 2.2*	9.6 \pm 2.3
Adiponectin	(μ g/mL)	15.7 \pm 1.3	14.2 \pm 1.0	13.7 \pm 1.1

Data are means \pm standard error (eight to nine mice per group).

* $p < 0.05$.

** $p < 0.01$ vs. low-fat diet (LFD) group.

*** $p < 0.05$ vs. HFD control group (*t*-test).

GIP, gastric inhibitory polypeptide; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NEFA, non-esterified free acid; T-Chol, total cholesterol; TG, triacylglycerol.

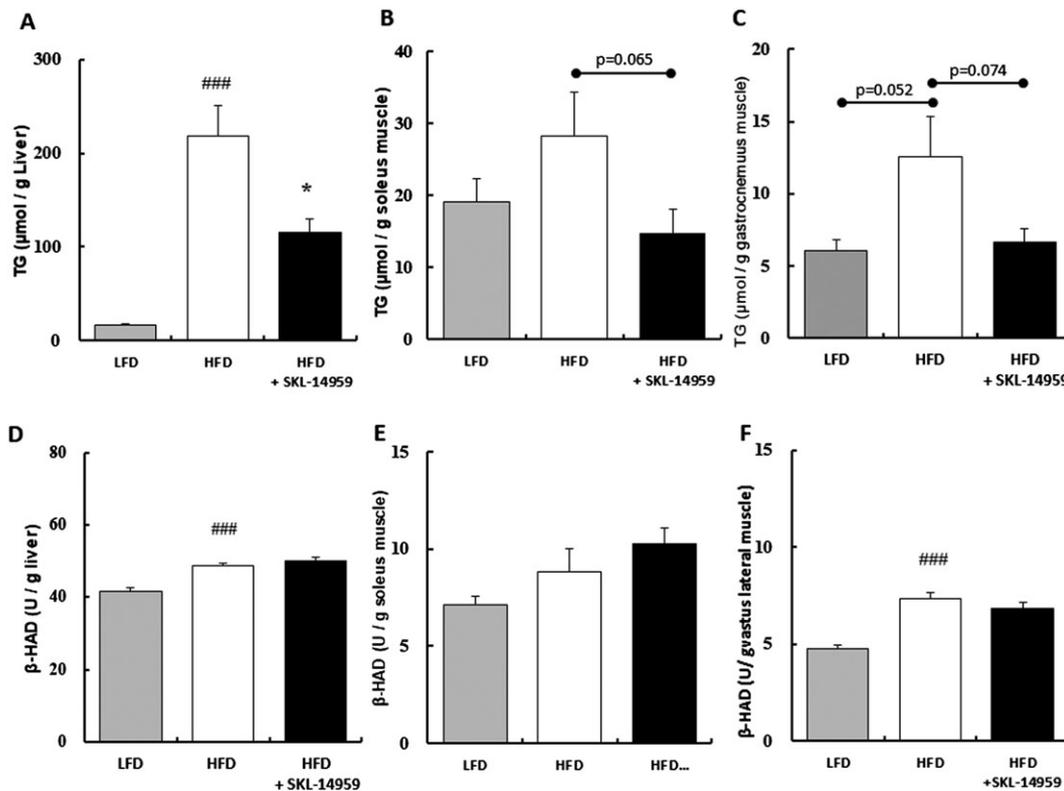


Figure 2 Effects of SKL-14959 on triacylglycerol (TG) contents in the liver (A), soleus muscle (B), and gastrocnemius muscle (C), and β -hydroxyacyl-CoA dehydrogenase (β -HAD) activity in the liver (D), soleus muscle (E) and vastus lateralis muscle (F). Data are means \pm standard error (eight to nine mice per group). ### $p < 0.001$ vs. LFD group, * $p < 0.05$ vs. HFD control group (t-test).

regulation of SREBP-1 (*Serbp1*, $p < 0.05$), but not PPAR-alpha (*Ppara*), compared with the HFD control group (Figure 4A). Moreover, the SKL-14959-treated group also indicated significant down-regulation of the relative gene expression of DGAT-1 (*Dgat1*, $p < 0.001$) in the final step of TG production, but the relative gene expression of FAS (*Fasn*) and ACC1 (*Acaca*) did not change (Figure 4A). On the other hand, the relative gene expression in the adipose tissue did not significantly alter relative to the HFD control group (Figure 4B).

Lipid distribution after a single administration of [14 C]-oleic acid

To investigate the excursion of ingested lipids on SKL-14959, [14 C]-oleic acid was acutely administered. SKL-14959 significantly decreased the radioactivity in the adipose tissues at 120 min in contrast with control ($p < 0.05$), whereas it did not evidence a significant difference in the liver and the muscle (Figure 5).

Discussion

Weight loss have resulted from either suppression of energy intake or increase of the energy expenditure or both.

From the viewpoint of energy consumption, the SKL-14959-treated group did not significantly enhance β -HAD activity in the liver and the muscle (Figure 2D–2F) nor significantly up-regulate the relative gene expression of PPAR-alpha (*ppara*) in the liver and the adipose tissue, UCP-2 (*ucp2*) and hormone sensitive lipase (*Lipe*) in the adipose tissue compared with the HFD control group (Figure 4A and 4B). It might be because GLP-1 made up for the effect (31); alternatively, raised β -HAD activity in the HFD control group compared with the LFD group was already reached maximal effectiveness, so that further combustible effects of SKL-14959 were not observed (32). These results suggested that SKL-14959 might not have direct action or have only a subtle effect of lipolysis.

From the viewpoint of energy intake, the processing of lipids anabolism using exogenous lipids, [14 C]-oleic acid, was evaluated, and the radioactivity increased time dependently until 6 h after gavage in the preliminary study (data not shown), suggesting that evaluated points in the present study could reflect the processing anabolic state. The SKL-14959-treated group indicated suppression of the radio activity in the adipose tissues but not in the liver and the muscle, contrast to the vehicle group 2 h after oil challenge (Figure 5). According to gene analysis of lipogenesis in the liver and the adipose tissue, the

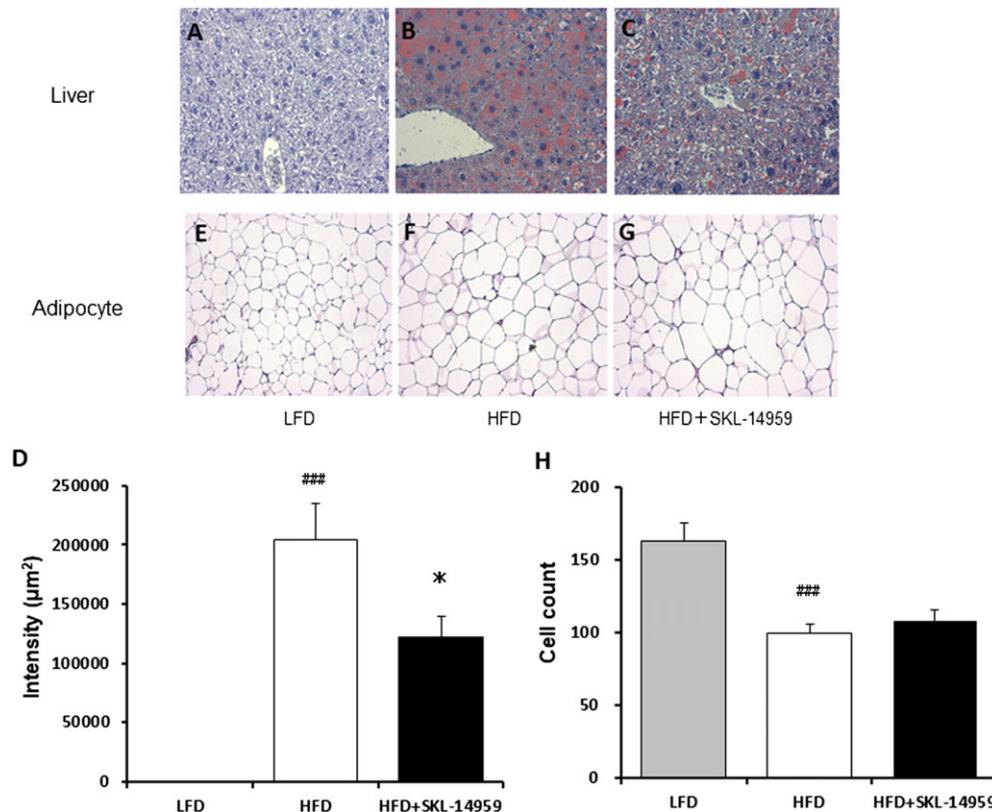


Figure 3 Histological analysis of SKL-14959 treatment on lipid droplets in the liver (A–D) and on the size in the epididymal adipocytes (E–H) represented mice in each group (eight to nine mice per group). Data are means \pm standard error (eight to nine mice per group). ### $p < 0.001$ vs. LFD group, * $p < 0.05$ vs. HFD control group (*t*-test).

SKL-14959-treated group showed significant down-regulation relative gene expression of SREBP1 (*srebp1*) and DGAT1 (*dgat1*) in the liver (Figure 4A) compared with the HFD control group but did not vary in the adipose tissue (Figure 4B). Moreover, lipid contents in the liver and muscle of the SKL-14959-treated group dropped to 45% and that level of the HFD control group, respectively (Figure 2). However, there is no report that GIP receptor expresses in liver and muscles in rodents (33,34). Thus, that suggested that the suppression of body weight gain and lipid accumulation might be not direct, but indirect effect of GIP receptor signalling especially could be secondary to reduced insulin release (29). We also had reported that SKL-14959 trended to decrease LPL activity in post-heparin plasma (25). This enzyme is secreted by adipose and muscles, which is anchored to the surface of endothelial cell and hepatic sinusoids. Plasma LPL binding to extracellular proteoglycan has associated with lipid intake and the control of lipid metabolism in remote organs. NEFA is a triglyceride degradation product by lipase, which is incorporated into cell through the transporter. Thus, these results suggested that SKL-14959 inhibited the incorporation of fatty acid into the adipocytes, the liver and the muscle by suppressing LPL

activity, and glucose utilization is likely to promote in the liver and the muscle by energy depletion.

Actually, the SKL-14959-treated group trended to decline the plasma ketone bodies level compared with the HFD control group (Table 1). Ketone bodies in blood are biomarker of energy source instead of diminished glucose utilities in diabetic hyperglycaemia (35,36), which are high levels in individuals with diabetic hyperglycaemia (36) or in obese control relative to lean in DIO rat (37). Thus, this result suggested that SKL-14959 might improve the glucose metabolism in peripheral tissues, resulted in decrease lipid deposition. Therefore, SKL-14959 might indicate prominent effects in the liver and the muscle, not adipocyte, and might have the suppressive effect of ectopic lipid deposition in systemic body. It is possible that SKL-14959 might also have the anti-diabetic effects.

In histological analysis, the SKL-14959-treated group did not affect the size of adipocytes compared with the HFD control group (Figure 3). In the present study, this phenotype is consistent with the result that plasma concentrations of adiponectin secreted from the adipocytes in inverse proportion to its size. Okuno et al. reported (38) that troglitazone increased the number of small adipocytes in obese rat. These results suggested that

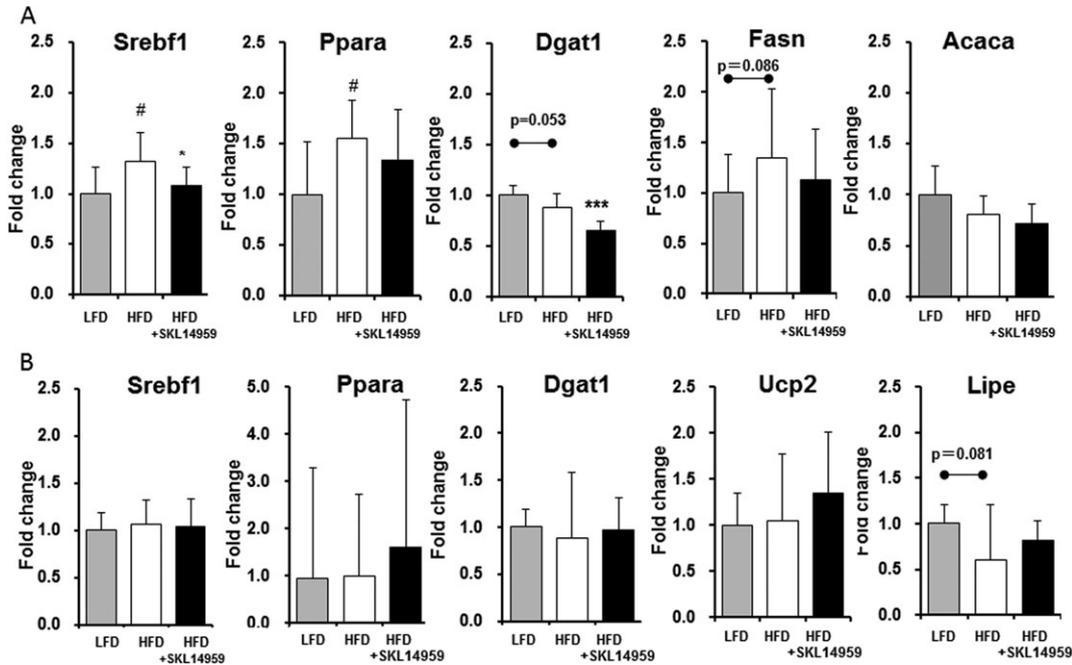


Figure 4 The relative gene expression of SREBP1 (*Srebf1*), PPAR-alpha (*Ppara*), DGAT-1 (*Dgat1*), FAS (*Fasn*) and ACC1 (*Acaca*) in the liver (A) and SREBP1 (*Srebf1*), PPAR-alpha (*Ppara*), DGAT-1 (*Dgat1*), UCP2 (*Ucp2*) and HSL (*Liipe*) in the adipose tissue (B) with regard to SKL-14959 treatment. Values are expressed as means ± standard deviation (eight to nine mice per group). # $p < 0.05$ vs. LFD group (*t*-test), * $p < 0.05$ vs. HFD control group (*t*-test).

SKL-14959 did not possess the potency of adipocyte proliferation such as peroxide-per gamma agonists.

There are some limitations in the present study. The evaluation period was short. To elucidate the effect of SKL-14959 in the weight loss, glucose tolerance and insulin sensitivity, it would have done the long-term study. Another one, the effect of neither normal nor low fat diet was evaluated. Furthermore, it remains undetermined which agent is superior to another because SKL-14959 was not directly compared with sibutramine or lipase inhibitor in weight loss in the same study.

In the point of clinical implications, because the target of SKL-14959 is not central anorexiant, it may be low risk of psychiatric behaviour. Additionally, this compound is an oral agent, and patients can avoid all the trouble from getting injection.

In conclusion, SKL-14959, the GIP receptor antagonist, suppressed weight gain and lipids deposit in the liver and the muscle regardless of food intake and indirect action of lipolysis. Additionally, SKL-14959 tended to improve diabetes status by raising glucose availability in peripheral tissue and acutely inhibited lipids intake into

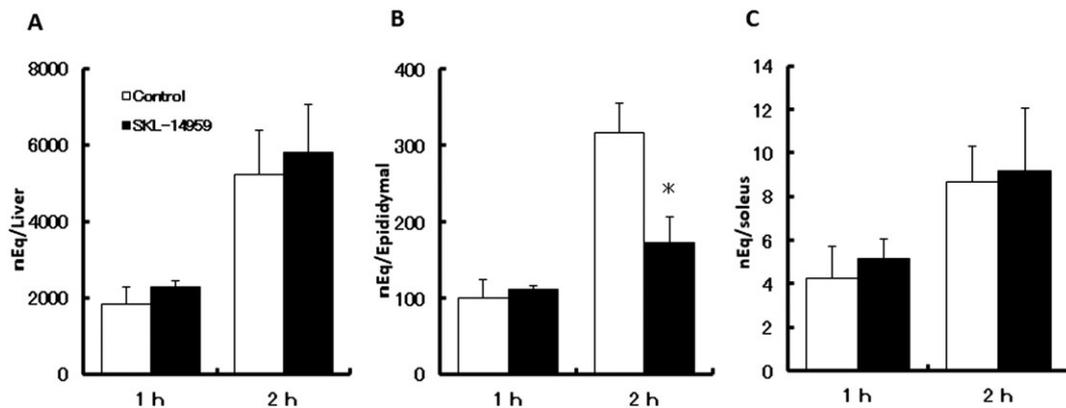


Figure 5 Effects of SKL-14959 on the radioactivity in liver (A), epididymal adipose tissue (B) and soleus muscle (C) after oral [¹⁴C]-oleic acid gavage. The 0.5% sodium carboxymethyl cellulose solution with or without SKL-14959 (100 mg / kg body weight) was orally administered 15 min prior to the oil challenge. Values are expressed as means ± standard error (four to six mice per group). * $p < 0.05$ versus control group (*t*-test).

the adipocyte. Thus, SKL-14959 has more effect on the inhibition of the lipid anabolism for calorie intake than on the enhancement of the lipid catabolism for energy expenditure that results in the long-lasting reduction of adiposity and suppression of weight gain. Therefore, it is possible that SKL-14959 may be a desirable new candidate for obesity and diabetic mellitus.

Conflict of interest statement

The authors declared no conflicts of interest.

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Author Contributions

H. Noda and Y. Tsubamoto conceived the study. H. Tanimoto, M. Okamoto and Y. Mizuno performed experiments. M. Takeuchi operated the gene analysis. We greatly appreciate the excellent technical assistance by Chika Ohtani and Mai Nampei. M. Okamoto reviewed the manuscript.

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