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ARTICLE

Anti-obesity Effect of Phosphatidylinositol on Diet-Induced Obesity in Mice

Kosuke Shimizu,[†] Tomoko Ida,[†] Haruhito Tsutsui,[†] Tomohiro Asai,[†] Kazumasa Otsubo,[‡] and Naoto Oku*,[†]

[†]Department of Medical Biochemistry and Global Center of Excellence (COE) Program, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan, and [‡]Asahi Kasei Pharma Corporation, 1-105 Kanda Jinbocho, Chiyoda-ku, Tokyo 101-8108, Japan

The aim of this study is to investigate the biodistribution of phosphatidylinositol (PI) after oral administration and its anti-obesity effect. When a suspension of radiolabeled PI was orally administered to mice and the biodistribution was examined, PI radioactivity accumulated in the liver compared to *myo*-inositol radioactivity at 48 h or later after administration. Then, a PI suspension was orally administered to diet-induced obesity (DIO) mice every 4 days, and the anti-obesity effect of PI was examined. As a result, PI suppressed the body weight increase of DIO mice and significantly reduced the plasma levels of aspartate aminotransferase (AST) and cholesterol. Furthermore, PI regulated the expression of some genes in the liver involved in lipid synthesis and metabolism. The present study demonstrated that PI accumulated in the liver after oral administration and exerted its anti-obesity effect on DIO by regulating the expression of certain genes involved in lipid metabolism in the liver.

KEYWORDS: Phosphatidylinositol; *myo*-inositol; biodistribution; liver; anti-obesity effect

INTRODUCTION

Because the prevention of common diseases promises a human life of health and longevity, many functional foods and supplements have been developed for this purpose. Phospholipids are abundant in food and are expected as the functional components for improvement of lifestyle-related diseases (*1*). Phospholipids representatively include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI), and these molecules mainly function as structural components of plasma membranes and subcellular organelle membranes. Among them, PI and its metabolites are known to function as not only components of the cell membrane but also second messengers in various signal transduction pathways (*2*), even though the PI content is less than 10% of the total content of phospholipids. For example, inositol 1,4,5-trisphosphate (IP_3) is known to be formed by the hydrolysis of a PI derivative, PI-4,5-bisphosphate (PIP_2), and plays an important role in regulating cell growth and maintenance (*3*−*5*). PI is a minor component as one of the phospholipids in most general foods, and even soybean, which is most abundant in PI of all food materials, contains only 287 mg/100 g as PI. However, several biological functions of exogenous PI are known. For example, PI inhibits vascular endothelial growth factor (VEGF)-induced angiogenesis *in vitro* (*6*), decreases the liver concentration of triacylglycerol (TG) in obesity-model Zuker$^{(fa/fa)}$ rats (*7*), promotes cholesterol transport and excretion (*8*), and suppresses amyloid β (Aβ)-induced degradation of hippocampal neurons of τ-expressing mice *in vivo* (*9*, *10*). On the other hand, there are also some reports about the function of *myo*-inositol (Inositol), a structural component of PI (*11*). Inositol is a sugar alcohol derived from PI or phosphorylated PI derivatives and is involved in cellular signal transduction. Inositol is one of the most active forms of all inositols and shows several functions, such as the prevention of fatty liver disease (*12*, *13*) and the improvement of panic disorder (*14*). These facts further indicate that PI may have the possibility to serve as a multifunctional component against common diseases.

In the present study, we first investigated the absorption and biodistribution of PI after oral administration in comparison to those parameters of Inositol. Then, the anti-obesity effect of PI was investigated using diet-induced obesity (DIO) mice. Finally, to elucidate the mechanism of the anti-obesity effect of PI, we examined gene expression changes in the liver of DIO mice after PI administration using DNA microarray analysis.

MATERIALS AND METHODS

Materials. Highly purified phosphatidylinositol (48.1%) was the product of Asahi Kasei Pharma Corporation. Inositol was obtained from Wako Pure Chemical Industries, Ltd., and phosphatidylinositol having L-α-[*myo*-inositol-2-^{3}H (N)] and *myo*-inositol-2-^{3}H (N) were purchased from Perkin−Elmer Co., Ltd. and American Radiolabeled Chemicals, Inc., respectively. PI suspensions were prepared by adding PI to a solution of 5 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer (pH 7.4) containing 1 mM ethylenediaminetetraacetic acid (EDTA)

*To whom correspondence should be addressed: Department of Medical Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka City, Shizuoka 422-8526, Japan. Telephone: +81-54-264-5701. Fax: +81-54-264-5705. E-mail: oku@u-shizuoka-ken.ac.jp.

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and stirring the mixture at room temperature overnight. Inositol solution was prepared in a similar manner.

Animals. Male, 5-week-old, BALB/c and C57BL/6J mice were purchased from Japan SLC, Inc. and Charles River Laboratories Japan, Inc., respectively. DIO mice were established by *ad libitum* intake of a high-fat (HF) diet (D12492, 60 kcal % fat, Research Diets, Inc.) instead of the normal-fat (NF) diet (D12450B, 10 kcal % fat, Research Diets, Inc.) by C57BL/6J mice. Animal care and experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the University of Shizuoka.

Biodistribution of PI after Oral Administration. [^{3}H]-Labeled PI ([^{3}H]PI) was suspended with unlabeled PI (Asahi Kasei Pharma Corporation), and the concentration of PI was adjusted to 120 mg kg^{-1} mouse^{-1} (74 kBq/mouse) for oral administration. [^{3}H]-Labeled Inositol ([^{3}H]Inositol) was mixed with a solution of non-labeled Inositol (Wako Pure Chemical Industries, Ltd.), and the concentration of Inositol was adjusted to be the same molar concentration as that of PI. Before the biodistribution study, BALB/c mice were starved overnight. [^{3}H]PI suspension or [^{3}H]Inositol solution was orally administered to the mice, and at 3, 24, 48, 72, and 96 h after administration, 5 mice per time point were sacrificed under anesthesia with diethyl ether to collect the plasma and organ samples, namely, brain, heart, lungs, liver, stomach, spleen, and kidneys. The accumulation of radioactive compound derived from [^{3}H]PI or [^{3}H]Inositol in each organ was determined with a liquid scintillation counter (Aloka, LSC-3100). Distribution data were presented as the percentage of injected dose per tissue. The total weight of plasma was assumed to be 4.38% of the body weight.

Biodistribution of PI after Intravenous Injection. [^{3}H]PI was mixed with a suspension of unlabeled PI (Sigma-Aldrich Co.), and the concentration was adjusted to 2.4 mg kg^{-1} mouse^{-1} (40 kBq/mouse). Then, [^{3}H]PI suspension or [^{3}H]Inositol solution was intravenously injected into the mice via a tail vein. At 3, 24, 48, and 72 h after the injection, 5 mice per time point were sacrificed under anesthesia with diethyl ether and then the plasma and organs were collected for the measurement of radioactivity.

Determination of PI Form in Plasma. [^{3}H]PI mixed with a suspension of unlabeled PI or [^{3}H]Inositol mixed with a solution of unlabeled Inositol (Wako Pure Chemical Industries, Ltd.) was orally administered to the mice (74 kBq/mouse). At 1 and 3 h after administration, 4 mice per time point were sacrificed under anesthesia to collect the blood. After centrifugation of the blood (900*g*, 10 min, 4 °C), the supernatant was diluted with phosphate-buffered saline (PBS). Separation of PI into organic and aqueous fractions was performed as follows according to a modification of the Bligh−Dyer method: the sample (900 μL) was added to 2 mL of methanol and 1 mL of chloroform and mixed at 50 °C. After incubation at room temperature for 10 min, 2 mL of chloroform and 1 mL of PBS were added to the mixture, which was then vigorously vortexed at 50 °C. Then, the sample was centrifuged (220*g*, 5 min) to separate aqueous, interface, and organic fractions. The radioactivity of each fraction was measured using a liquid scintillation counter (Aloka, LSC-3100).

Anti-obesity Effect of PI on DIO Mice. DIO mice were prepared by providing the HF diet *ad libitum* to C57BL/6J mice for 21 days starting from the age of 6 weeks. Then, Inositol solution (24.3 mg kg^{-1} day^{-1}, same as the mole dose of PI, Wako Pure Chemical Industries, Ltd.) or PI suspension (120 mg kg^{-1} day^{-1} as PI dosage, Asahi Kasei Pharma Corporation) was orally administered to the DIO mice every 4 days. The control group was administered 0.2 mL of 5 mM HEPES buffer solution (pH 7.0) containing 1 mM EDTA. The body weight of each mouse was monitored every 2 days for the evaluation of the anti-obesity effect. At day 50 after starting the administration of samples, the mice were sacrificed under anesthesia with diethyl ether and blood samples were collected. The levels of lactose dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), free cholesterol (F-CHO), total cholesterol (T-CHO), triglycerol (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and uric acid (UA) in the plasma were measured by a biochemical analyzer (7020 Clinical Analyzer, Hitachi High-Technologies Corporation, Tokyo, Japan).

DNA Microarray. PI suspension (120 mg kg^{-1} day^{-1} as PI dosage, Asahi Kasei Pharma Corporation) or HEPES buffer was orally administered to DIO mice every day for 1 week (from days 35 to 41). Then, their livers were excised 24 h after the last administration, and the total RNA in the liver was extracted using an RNeasy Plus Mini Kit (QIAGEN). The samples for DNA microarray analysis were prepared in accordance with the manual of Whole Mouse Genome OligoDNA Microarray Kit (Agilent Technologies, Inc.) and scanned with an Agilent DNA microarray scanner. Each spot on the microarray was imaged, and the data were calculated using Feature Extraction Software (Agilent Technologies, Inc.) and analyzed by GeneSpring GX Software (Agilent Technologies, Inc.).

Statistical Analysis. The variance in a group was evaluated using the *F* test, and the difference between two groups were evaluated using Student's *t* test or the Wilcoxon−Mann−Whitney test.

RESULTS

Biodistribution of PI. We first investigated the biodistribution of [^{3}H]PI after oral administration to BALB/c mice and the difference in biodistribution between radioactive compounds of PI and Inositol, which is a part of the PI structure. As a result, more than 10% of the administered radioactivity of PI or Inositol was absorbed into the mouse body by 3 h after the administration, and either radiolabel was distributed throughout the body, especially in the kidneys, liver, and brain (**Figure 1A**). The amount of PI radioactivity in the blood was about 2% of the injected dose at 3 h, and about 1% of the radiolabel was retained even at 96 h (**Figure 1B**). The biodistribution pattern of PI radioactivity was similar to that of the Inositol one, except that there was higher accumulation of Inositol than PI radioactivity in the kidneys. On the other hand, at 48 h or later, the radioactivity of PI significantly accumulated in the liver in comparison to that of Inositol (panels **C** and **D** of **Figure 1**). These results suggest that PI effectively accumulated in the liver after oral administration and that the accumulated PI was retained for a longer time in comparison to the accumulated Inositol.

To elucidate the reason why PI accumulated in the liver to a greater degree than Inositol after oral administration, we next investigated the biodistribution of [^{3}H]PI or [^{3}H]Inositol in mice after intravenous injection via a tail vein. The results indicated that the biodistribution of PI radioactivity at 3 h was far different from that of Inositol and that almost all of the PI had accumulated in the liver (**Figure 2A**). This higher accumulation of PI in the liver was retained up to 96 h (**Figure 2B**). In addition, the blood concentration of PI radioactivity was significantly higher than that of Inositol radioactivity at 3 h (**Figure 2A**). These results suggest that PI in the bloodstream had a longer circulating potential than Inositol and was likely to be delivered to the liver.

Determination of PI in Plasma after Oral Administration. Although the distribution of radioactive compounds after the oral administration of PI and Inositol was quite similar, suggesting that almost all of the PI was degraded during absorption, we hypothesized that part of the PI was absorbed as its intact form, because PI radioactivity in the liver was higher than that of Inositol. Thus, we investigated to confirm the presence of radioactivity in the lipidic fraction when blood was collected after oral administration of [^{3}H]PI. At first, we confirmed that almost all of the [^{3}H]PI radioactivity was transferred to the organic fraction, whereas almost all of the [^{3}H]Inositol radioactivity was detected in the aqueous fraction, when they were directly added to plasma and separated by the Bligh−Dyer extraction method (data not shown). Then, [^{3}H]PI or [^{3}H]Inositol was administered to mice, and after 3 h of administration, the plasma was collected. Thereafter, the plasma was separated into organic and aqueous fractions using the Bligh−Dyer extraction method, and the radioactivity in each fraction was measured. As shown in **Figure 3**, part of the radiolabeled form was found in the organic layer, when [^{3}H]PI was administered. On the contrary, when [^{3}H]Inositol was administered, almost all of the radioactivity was present in the

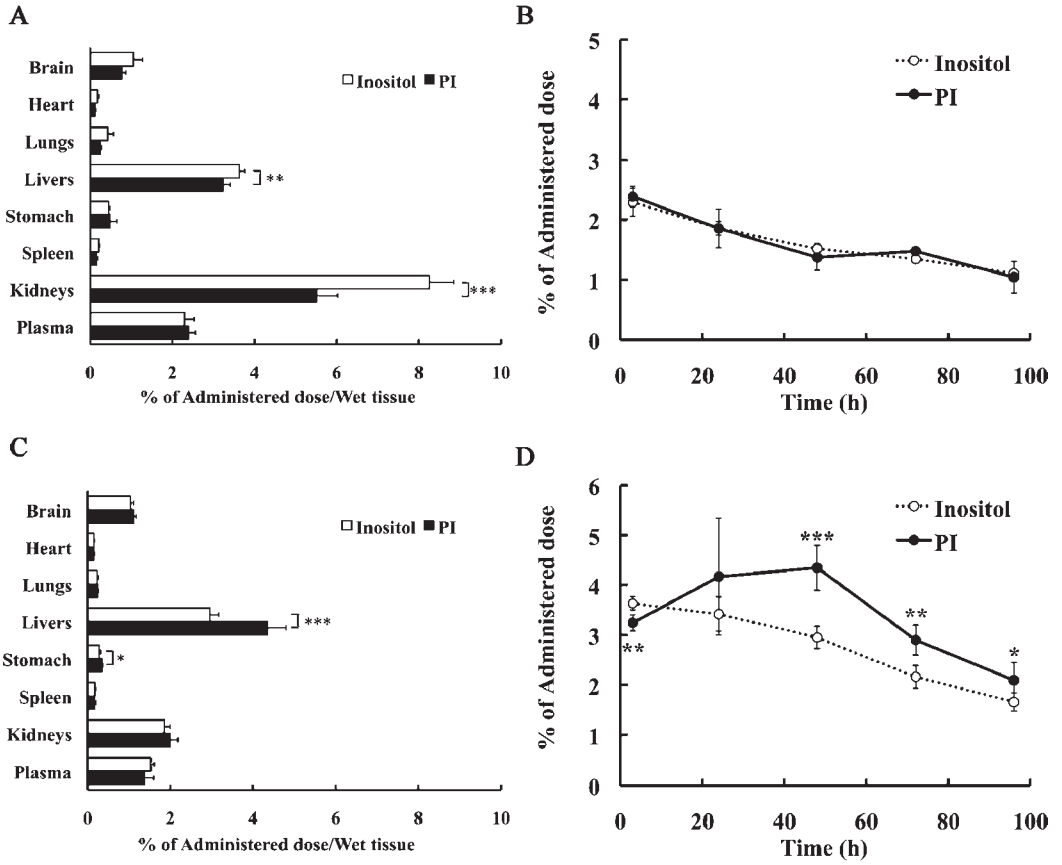

Figure 1. Biodistribution of PI and Inositol after oral administration. [^{3}H]-Labeled PI suspension (120 mg/kg) or [^{3}H]-labeled Inositol solution was orally administered to BALB/c mice ($n = 5$). The mice were sacrificed under diethyl ether anesthesia, and their organs were collected at 3, 24, 48, 72, and 96 h after administration. Then, the radioactivity in each organ was determined. The biodistribution patterns of PI and Inositol at (A) 3 h and (C) 48 h after administration was shown. Data are presented as a percentage of the administered dose per wet tissue. Error bars represent standard deviation (SD). Concentration profiles of PI and Inositol in the (B) plasma and (D) liver are also shown. Significant differences from Inositol are indicated (Student's t test: ∗, $p < 0.05$; ∗∗, $p < 0.01$; ∗∗∗, $p < 0.001$). Similar results were obtained in a separate experiment.

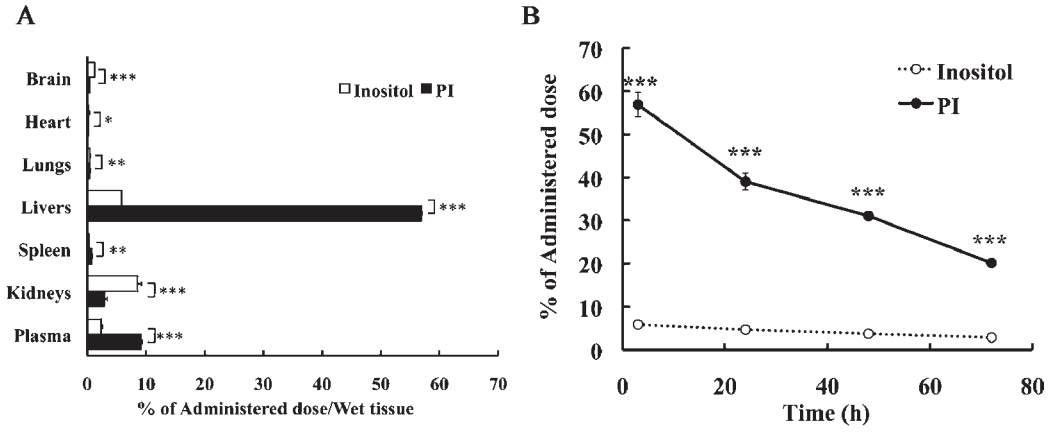

Figure 2. Biodistribution of PI and Inositol after intravenous injection. [^{3}H]-Labeled PI suspension (0.27 mM) or Inositol solution (0.2 mL) was intravenously injected into BALB/c mice ($n = 5$) via a tail vein. The mice were sacrificed under diethyl ether anesthesia, and their organs were collected at 3, 24, 48, 72, and 96 h after the injection. Then, the radioactivity in each organ was determined. (A) Biodistribution patterns of PI and Inositol at 3 h after the injection are shown. Data are presented as a percentage of the administered dose per wet tissue. Error bars represent SD. Concentration profiles of PI and Inositol in the (B) liver are also shown. Significant differences from Inositol are indicated (Student's t test: ∗, $p < 0.05$; ∗∗, $p < 0.01$; ∗∗∗, $p < 0.001$). Similar results were obtained in a separate experiment.

aqueous layer and at the interface. A similar result was found at 1 h after administration (data not shown). These results suggest that part of the orally administered PI had retained its hydrophobic character, i.e., its intact form, in the bloodstream.

Anti-obesity Effect of PI on DIO Mice. Because the results obtained as described above suggest that part of the orally administered PI was delivered intact to the liver, we next examined the effect of PI on liver function. To examine the anti-obesity effect of

PI, we first prepared DIO mice, which ingested the HF diet every day *ad libitum*. As shown in **Figure 4A**, the body weight of these mice gradually increased in comparison to that of the normal mice fed the NF diet. Then, the PI suspension or Inositol solution was administered orally every 4 days starting from day 21 on the diet, and the body weight of these DIO mice was observed. As a result, the body weight of PI-administered DIO mice on day 39 or later was significantly lower than that of the control DIO mice, which had been administered buffer alone (**Figure 4B**). In addition, PI did not change the body weight of the mice given the NF diet (data not shown). Inositol also suppressed the body weight increase caused by intake of the HF diet, although the suppressive effect was not so strong compared to that achieved by PI (**Figure 4B**). There were no differences in the amount of food intake among the three groups (**Figure 4C**).

Additionally, we measured the level of biomedical values, such as hepatic injury markers, lipids, and uric acid in the plasma. PI administration resulted in the improvement of the plasma concentrations of LDH, AST, F-CHO, T-CHO, LDL-C, and uric acid in the DIO mice (**Figure 5**). Inositol administration also resulted in a reduction in these levels, although PI was more effective than Inositol in the case of LDH, AST, and uric acid.

PI-Induced Changes in Gene Expression in the Liver. To elucidate the mechanism of improvement of hepatic function by PI, we investigated possible changes in gene expression in the liver. PI suspension or buffer solution was orally administered to DIO mice, and the difference in gene expression in the liver between the two groups was assessed by DNA microarray analysis. The tables showed the genes that were upregulated (**Table 1**) or downregulated (**Table 2**) more than 3-fold by PI administration.

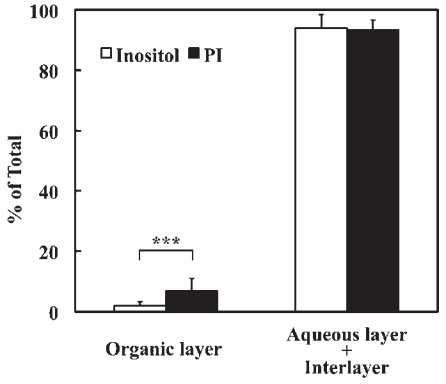

Figure 3. Separation of blood [^{3}H]PI into organic and aqueous layers. [^{3}H]-Labeled PI or Inositol (120 mg/kg) was orally administered to BALB/c mice (n = 4). Blood samples were collected from the mice under diethyl ether anesthesia at 3 h after administration. Then, the plasma samples were separated into organic, interface, and water layers using the Bligh−Dyer extraction method. Then, each layer was collected, and the radioactivity was determined. Data are presented as a percentage of the total extracted radiolabeled form from plasma samples. Error bars represent SD. Significant differences are shown (Student's t test: ∗∗∗, $p < 0.001$, as indicated by the bracket). Similar results were obtained in a separate experiment.

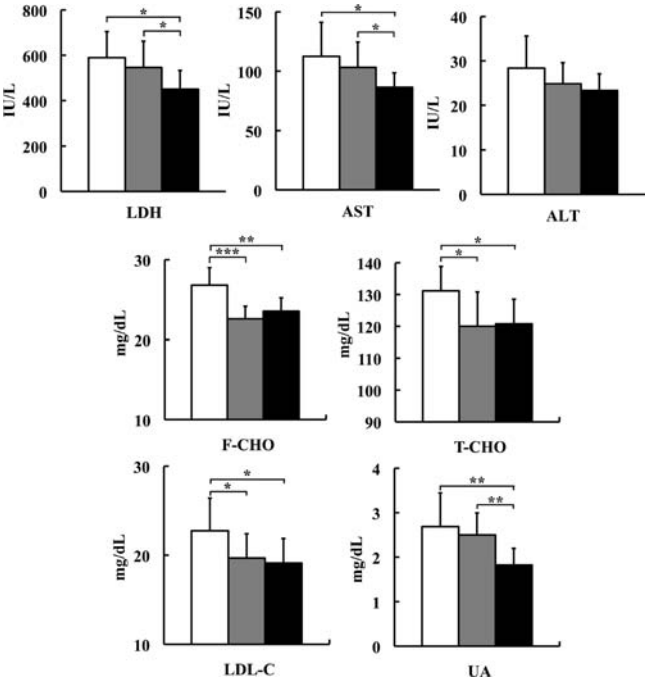

Figure 5. Effect of PI on plasma levels of liver enzymes, lipids, and uric acid. On day 50 after the start of HF diet intake, blood samples from buffer- (white bar), Inositol- (gray bar), or PI- (black bar) administered DIO mice (n = 8 or 9) were collected after the animals had fasted for 16 h. The plasma samples were analyzed using a biochemical analyzer (7020 Clinical Analyzer, Hitachi High-Technologies Corporation, Tokyo, Japan). Data represent the mean value and SD. Significant differences are shown (Wilcoxon−Mann−Whitney test: ∗, $p < 0.05$; ∗∗, $p < 0.01$; ∗∗∗, $p < 0.001$, as indicated by brackets. Similar results were obtained in a separate experiment.

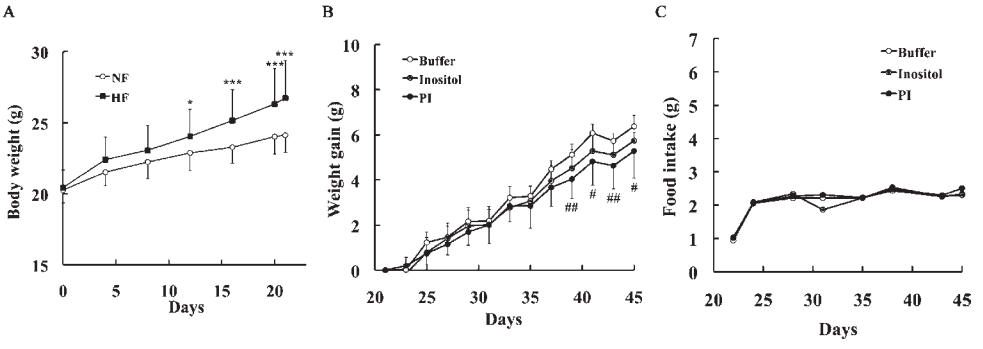

Figure 4. Effect of PI on body weight increase in DIO mice. NF diet or HF diet was given to C57BL/6J mice *ad libitum* to prepare the DIO mice (n = 8 or 9). (A) When a significant difference in body weight between the two groups was observed (day 21), oral administration of PI suspension (120 mg kg^{-1} day^{-1}, black circle), Inositol (120 mg kg^{-1} day^{-1}, gray circle) or HEPES buffer (white circle) was started (every 4 days). (B) Body weight change from day 21 was calculated, and (C) amount of daily food intake was measured. Error bars represent SD. Significant differences are indicated (Wilcoxon−Mann−Whitney test: ∗, $p < 0.05$; ∗∗∗, $p < 0.001$ versus NF; #, $p < 0.05$; ##, $p < 0.01$ versus buffer). Similar results were obtained in a separate experiment.

Table 1. Upregulated Genes in the Liver of DIO Mice by PI Administration

common name	gene symbol	fold change	description	gene ontology
AK081660	AK081660	4.67	*Mus musculus* 16 days embryo head cDNA, RIKEN full-length enriched library, clone: C130061P18 product: unclassifiable, full insert sequence	
AK053661	Exosc5	4.62	*Mus musculus* 0 day neonate eyeball cDNA, RIKEN full-length enriched library, clone: E130119H13 product: unclassifiable, full insert sequence	rRNA processing
NM_178372	Prss34	4.40	*Mus musculus* protease, serine, 34, mRNA	proteolysis
NM_026250	Zh2c2	3.81	*Mus musculus* zinc finger, H2C2 domain containing, mRNA	DNA recombination; DNA integration
ENSMUST00000062223	ENSMUST00000062223	3.76	mitochondrial carrier homologue 2 (*Caenorhabditis elegans*) [source: MarkerSymbol; Acc: MGI:1929260]	mitochondrial inner membrane
AK079356	9630041G16Rik	3.63	*Mus musculus* 16 days neonate cerebellum cDNA, RIKEN full-length enriched library, clone: 9630041G16 product: unclassifiable, full insert sequence	
NM_133872	Aof2	3.29	*Mus musculus* amine oxidase (flavin containing) domain 2, mRNA	electron transport; transcription; regulation of transcription, DNA dependent; chromatin modification
AK142069	Socs6	3.15	*Mus musculus* 12 days embryo eyeball cDNA, RIKEN full-length enriched library, clone: D230020G23 product: suppressor of cytokine signaling 6, full insert sequence	regulation of cell growth; cell glucose homeostasis; intracellular signaling cascade; negative regulation of signal transduction; regulation of growth
NM_146144	Usp1	3.02	*Mus musculus* ubiquitin specific peptdiase 1, mRNA	ubiquitin-dependent protein catabolic process
NM_025572	2610528J11Rik	3.02	*Mus musculus* RIKEN cDNA 2610528J11 gene, mRNA	membrane; integral to membrane

Table 2. Downregulated Genes in the Liver of DIO Mice by PI Administration

common name	gene symbol	fold change	description	gene ontology
NM_009780	C4b	3.01	*Mus musculus* complement component 4B (Childo blood group), mRNA	complement activation; immunoglobulin-mediated immune response
NM_012055	Asns	3.06	*Mus musculus* asparagine synthetase, mRNA	asparagine biosynthetic process; glutamine metabolic process; metabolic process; amino acid biosynthetic process
NM_011315	Saa3	3.50	*Mus musculus* serum amyloid A 3, mRNA	acute-phase response (lipid transporter activity)
NM_009117	Saa1	3.78	*Mus musculus* serum amyloid A 1, mRNA	acute-phase response (lipid transporter activity)
NAP123621-1	NAP123621-1	3.89		
NM_009127	Scd1	4.13	*Mus musculus* stearoyl-CoA desaturase-1, mRNA	lipid metabolic process; fatty acid biosynthetic process; superoxide metabolic process; lipid biosynthetic process
NM_013459	Cfd	4.62	*Mus musculus* complement factor D (adipsin), mRNA	proteolysis; immune response; complement activation, alternative pathway; innate immune response
AK018789	Ntrk2	5.39	*Mus musculus* adult male cerebellum cDNA, RIKEN full-length enriched library, clone: 1500040I13 product: neurotrophic tyrosine kinase, receptor, type 2, full insert sequence	vasculogenesis; protein amino acid phosphorylation; transmembrane receptor protein tyrosine kinase signaling pathway; multicellular organismal development; nervous system development; cell differentiation; mechanoreceptor differentiation; retinal rod cell development

Notably, PI upregulated the expression of the suppressor of cytokine signaling-6 (Socs6) gene, which is involved in the improvement of insulin resistance (**Table 1**). On the other hand, it downregulated the gene expressions of neurotrophic tyrosine kinase receptor-2 (Ntrk2), stearoyl-CoA desaturase-1 (Scd 1), serum amyloid A1 (Saa1), and serum amyloid A3 (Saa3), all of which are involved in lipid metabolism (**Table 2**). These results suggest that PI potentially regulated the expression of various genes in the DIO mice, resulting in the suppressive effect on the DIO.

DISCUSSION

With recent discoveries of the risk factor for common diseases, such as obesity, hypertension, and diabetes, concern about the human diet has been rising. Coincident with this, functional components in the daily diet have been a matter of focus, and mechanistic studies on them, for improvement of common diseases, are ongoing. PI, a kind of phospholipid, has been of interest, owing to its multifunctional characteristics, and is expected to be applied as a functional food for human health and longevity (*3*). However, the absorption, biodistribution, and definite function of PI after intake are largely unclear.

In this study, we attempted to elucidate the potency of PI against DIO. We first investigated the absorption of PI into the body of mice after oral administration and the subsequent biodistribution. More than 10% of the administered PI radioactivity had already been distributed throughout the body by 3 h after administration (**Figure 1A**), indicating that PI was efficiently absorbed into the body. Inositol showed a similar biodistribution of PI radioactivity, except for its greater accumulation in the kidneys, and the blood concentration was almost the same as that of PI radioactivity up to 96 h (**Figure 1B**). These results suggest that most of the PI was converted to Inositol in the process of transfer to the bloodstream, resulting in a biodistribution similar to that of Inositol. It is generally known that orally administered phospholipids are degraded by phospholipases and lipases,

absorbed into the lymphatic vessels of the small intestines, and then transferred to the bloodstream. However, it is also known that certain phospholipids are transferred to the bloodstream without complete degradation. Galli et al. previously reported that dilinoleoyl-PC double-radiolabeled with both ^{3}H at its choline and ^{14}C at its fatty acid moieties accumulates in erythrocyte membranes and in lipoproteins, such as HDL and LDL, after oral administration and shows prolonged circulation in the bloodstream, because a part of the PC is converted to its lyso product in the process of systemic absorption (*15*). This report supports our data that PI was partly absorbed into the bloodstream without complete degradation. Indeed, PI significantly accumulated in the liver compared to Inositol at 48 h or later after administration (**Figure 1C**).

To clarify the reason for this difference in accumulation, we next compared the biodistribution between PI and Inositol radioactivity after intravenous injection. As to the accumulation in the liver, PI radioactivity accumulated in the liver to a much greater extent compared to that of Inositol (**Figure 2**), suggesting that PI circulating in the bloodstream as phospholipid or its lyso product form had the potential for transfer to the liver.

To demonstrate the validity of this speculation, we investigated the presence of radiolabeled lipidic compounds in the bloodstream after oral administration of [^{3}H]PI. As the result of analysis of the plasma sample collected at 3 h after administration, the radioactivity in the organic fraction was significantly higher in the sample from the PI-administered mice, whereas almost all of the [^{3}H]Inositol radioactivity was detected in the aqueous fraction (**Figure 3**). This result suggests that part of the PI after oral administration was transferred to the systemic bloodstream without structure change and accumulated in the liver.

Concerning the functional effect of PI against common diseases, we prepared obesity mice, which completely reflected the human obesity and are largely used in the research field of obesity, and examined the effect of orally administered PI on this DIO. PI administration significantly suppressed the body weight increase of the mice, whereas the suppression in the group given Inositol was less potent (**Figure 4B**). Additionally, when the levels of various components in the plasma of the DIO mice were examined, those of LDH, AST, F-CHO, T-CHO, and LDL-C, which are indicators of liver function disorder, were significantly improved by the PI administration (**Figure 5**). Furthermore, the plasma level of uric acid, which has recently been used as an indicator of metabolic syndrome (*16*), was also decreased by the PI administration (**Figure 5**). These results suggest that PI suppressed the body weight increase of DIO mice by improving the liver function disorders caused by intake of the HF diet and reducing the amount of excess body fat. Shirouchi et al. previously reported that PI prevents the increase in the amount of TG seen in the liver of Zucker$^{(fa/fa)}$ rats, which have genetically induced fatty liver disease (*7*, *17*). In our study, PI did not decrease the amount of TG in the plasma of the DIO mice (data not shown), although other indicators of liver function were improved. This different result may have been caused by the amount of PI administered; i.e., the above investigators prepared PI mixed with daily feed and gave it to Zucker$^{(fa/fa)}$ rats (*7*, *17*), resulting in PI intake much greater than in our case. Thus, the amount of TG may have been decreased in our experiment if the administration dosage had been increased.

Finally, to understand the anti-obesity effect of PI, we investigated the gene expression changes in the liver induced by PI administration. PI suspension was administered to DIO mice every day for 1 week, and the gene expression in the liver was compared to that for buffer-administered mice using DNA microarray analysis. Data analysis revealed differences in the expression of certain genes between the two groups. As shown in **Table 1**, the expression of Socs6 was upregulated by PI administration. There is much evidence implicating insulin resistance in a number of metabolic disorders, including obesity, and insulin receptor signaling in hepatic cells is important for not only glucose uptake and glycogen synthesis but also antilipolysis (*18*). Interestingly, this insulin-receptor-mediated signaling is mediated by phosphatidylinositol (3,4,5)-triphosphate (PIP_3) as a second messenger (*19*, *20*). On the other hand, the family of Socs proteins modulates this signal transduction through the binding to the insulin receptor and/or by targeting the insulin receptor substrate (IRS) for proteosomal degradation (*21*, *22*). Mooney et al. previously reported that Socs6 promotes insulin-dependent activation of Akt signaling through the direct binding to the insulin receptor, whereas other Socs proteins, such as Socs1 and Socs3, inhibit this signaling (*23*). Thus, these findings support our data, showing that PI administration attenuated the insulin-receptor-mediated signaling through the upregulation of Socs6 expression and promoted lipolysis in hepatic cells.

There were also some genes downregulated by PI administration (**Table 2**). Ntrk2, the most downregulated gene, encodes TrkB, which is known as a receptor for neurotrophins, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). BDNF and Ntrk2 (TrkB) signaling in the central nervous system (CNS) is well-known to be involved in sensation, memory, learning, etc. Kernie et al. previously found that mice lacking BDNF exhibit abnormality in eating behavior or locomotion, and they concluded that BDNF and TrkB signaling in the CNS controls food intake (hyperphagia) (*24*). Their evidence indicates that Ntrk2 signaling in the CNS is involved in the obesity (*25*−*27*). Kuroda et al. demonstrated that BDNF functions in the liver of Zucker$^{(fa/fa)}$ rats to improve their insulin resistance by affecting hepatic glucose flux (*28*). However, our data showed that Ntrk2, the gene encoding TrkB, the receptor for BDNF, was decreased by PI intake. Thus, further studies will be necessary to elucidate the involvement of Ntrk2 in hepatic function.

On the other hand, we showed that the expression of Scd1 was significantly decreased. This enzyme has been implicated in not only insulin resistance but also DIO when it is abnormally expressed at a high level (*29*−*32*). In addition, the expression of Saa1 and Saa3 was also decreased by PI administration. According to the report by Chiba et al., the expression of Saa1 in the liver and Saa3 in adipose tissue is significantly increased in obese mice (*33*). Furthermore, Saa is known to correlate with obesity and insulin resistance in humans, thus making it a candidate marker of metabolic syndrome (*34*, *35*).

Burgess et al. have demonstrated the anti-obesity function of PI that intravenously administered PI was efficiently incorporated into HDL-C and promoted the effects of HDL on both intravascular cholesterol storage and reverse cholesterol transport and suggested PI acts through a regulation of lipoprotein charge and cell signaling pathway to alter both vascular and cellular cholesterol homeostasis (*36*). Other previous reports also demonstrated that exogenous addition of PI altered the intracellular metabolism of Inositol and the following Inositol signaling (*37*). This information and the results in the present study can promise that PI exerts its anti-obesity effect by affecting the expression of certain genes involved in lipid synthesis and metabolism in the liver, which was mediated by PI signaling.

In conclusion, our present study demonstrated that PI was effectively absorbed into the body of rats after oral administration. Although most of the PI was degraded during absorption, some of it was not and accumulated in the liver. Furthermore, we showed that the potent anti-obesity effect of PI on HF DIO was

due to PI-mediated regulation of the expression of certain genes involved in lipid synthesis and metabolism in the liver. Thus, daily intake of soybean-derived PI or supplemental intake of an adequate amount of PI would be expected to improve DIO.

ABBREVIATIONS USED

Aβ, amyloid β; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BDNF, brain-derived neurotrophic factor; DIO, diet-induced obesity; F-CHO, free cholesterol; HDL-C, high-density lipoprotein cholesterol; HF, high fat; IP_3, inositol 1,4,5-trisphosphate; IRS, insulin receptor substrate; LDH, lactose dehydrogenase; LDL-C, low-density lipoprotein cholesterol; Inositol, *myo*-inositol; NF, normal fat; NGF, nerve growth factor; Ntrk2, neurotrophic tyrosine kinase receptor-2; PBS, phosphate-buffered saline; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIP_2, PI-4,5-bisphosphate; PS, phosphatidylserine; Saa1, serum amyloid A1; Saa3, serum amyloid A3; Scd 1, stearoyl-CoA desaturase-1; Socs6, suppressor of cytokine signaling-6; T-CHO, total cholesterol; TG, triacylglycerol; UA, uric acid; VEGF, vascular endothelial growth factor.

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