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Effects of Dietary Phytol on Glucose Uptake and Insulin Secretion in Vitro and in Vivo

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Abstract

Phytol, which is a product of chlorophyll degradation, is known to possess diverse biological functions, including anti-obesity and anti-diabetes. Long-term intake of phytol has beneficial effects on insulin resistance, obesity, and diabetes via improvement of lipid metabolism. However, the exact mechanisms involved and effects of immediate action such as single intake of phytol on postprandial hyperglyceamia remain poorly understood. In present study, the effects of phytol on glucose uptake and associated mechanisms were investigated both in vitro and in vivo. We found that phytol induced insulin secretion by RIN-5Fand directly stimulated glucose uptake via the activation of 5' adenosine-monophosphate-activated protein kinase (AMPK) and phospatidylinositol-3 kinase/AKTin L6 myo tubes. Phytol also significantly suppressed the increase of postprandial blood glucose levels via AMPK activation, not AKT, of skeletal muscle and improved the abnormal patterns of insulin secretion in obese mice. These findings confirm the glucose uptake mechanism, specifically in skeletal muscle, by intake of dietary phytol and the immediate effects of phytol's health promoting ability.

Keywords: Phytol, Postprandial hyperglycemia, Obesity, Insulin secretion, Glucose uptake

Abbreviations: AKT: Protein Kinase B; AMPK: 5' Adenosine-monophosphate-activated Protein Kinase; AUC: Areas Under the Curve; DMEM: Dulbecco's Modified Eagle's Medium; ELISA: Enzyme-linked Immunosorbent Assay; FBS: Fetal Bovine Serum; GLUT4: Glucose Transporter 4; HFD: High-fat Diet; IRS: Insulin Receptor Substrates; KHH: Krebs-Henseleit-Hepes; LFD: Low-fat Diet; NAD: Nicotineamide Adenine Dinucleotide; OGTT: Oral Glucose Tolerance Test; PI3K: Phosphatidylinositol-3 Kinase; PPARs: Peroxisome Proliferator-activated Receptors; RPMI: Roswell Park Memorial Institute; RXR: Retinoid-X-Receptor; SE: Standard Errors;T2D: Type 2 Diabetes

INTRODUCTION

Several lifestyle factors affect the incidence of type 2 diabetes (T2D), while obesity and weight gain dramatically increase the risk of developing this disease. As an individual becomes more obese, they enter a more insulin-resistant state, leading to impaired glucose tolerance that can potentially lead to the onset of T2D [1]. Humans with T2D have normal or high insulin levels, but tissues, such as the liver, skeletal muscle, and adipose tissue, become resistant to this insulin. The pancreas compensate for this by producing large amounts of insulin, which can result in impaired glucose transport into these tissue [2,3].

The skeletal muscle tissue accounts for up to 80% of insulin-mediated glucose uptake in the postprandial state. The insulin signaling pathway that leads to increased glucose uptake into muscle involves the binding of insulin to its receptor, phosphorylation of downstream insulin receptor substrates (IRS), and activation of phosphatidylinositol-3 kinase (PI3K) and protein kinase B (AKT), which promotes the translocation of glucose transporter 4 (GLUT4) from an intracellular pool to the plasma membrane [4]. In addition, 5′ adenosine-monophosphate-activated protein kinase (AMPK) also contributes to enhanced glucose uptake.

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This heterotrimeric ser/thr kinase responds to an increase in the cellular AMP/ATP ratio [5], and therefore, is activated by exercise/contraction, metformin, and thiazolidinedione [6,7]. Consequently, AMPK has become an attractive pharmacological target for the treatment of insulin resistance, obesity, and T2D.

Phytol (3,7,11,15-tetramethylhexaded-2-en-1-ol), is a plastidial isoprenoid that forms part of the chlorophyll molecule [8,9]. It has previously been shown that phytol is a precursor for vitamin E and vitamin K1 [10]. In addition, phytol is a type of phytochemical-a bioactive, non-nutrient compound that is found in plant foods such as fruits, vegetables, and grains. Many phytochemicals are potent effectors of biological processes and are able to influence disease risk via several complementary, overlapping mechanisms [11]. Because almost all phytosynthetic organisms contain chlorophyll, phytol is abundant in various plant foods, including green leafy vegetables, for example, spinach is known to contain 62 mg phytol per 100 g wet weight [12]. It has been suggested that chlorophyll is only partially digested, with the phytol moiety being released into the body. However, chlorophyllase, which catalyzes the hydrolysis of chlorophyll to form chlorophyllide and phytol, actually promotes chlorophyllide formation upon disruption of leaf cells or when it is artificially mistargeted to the chloroplast [13]. Therefore, it is possible that humans could readily digest the phytol that is contained in green leafy vegetables.

Previous animal studies have indicated that phytol may have anticancer and antoxidative effects [14,15]. In addition, long-term intake of phytol may help with the management of insulin resistance and metabolic disorders, which accompany diabetes and/or obesity, by activating the major regulators of lipid metabolism, including retinoid-X-receptor (RXR) and peroxisome proliferator-activated receptors (PPARs) [16,17]. We previously reported that phytol increased the level of human blood nicotineamide adenine dinucleotide (NAD), strongly linked to diabetes, in the rat liver [18,19]. However, the effects of immediate action such as single intake of phytol on postprandial hyperglyceamia via enhancing glucose uptake and insulin secretion have not previously been reported. Thus, the present study investigated the promotional effects of phytol on glucose uptake, its mechanisms in myotubes, insulin secretion in pancreatic β-cells, and the effects of a single administration of phytol on increases in postprandial blood glucose and insulin levels in obese mice fed a high-fat diet.

MATERIALS AND METHODS

In vitro studies

In vitro cell culture of RIN-5F and L6 myotubes: RIN-5F cells from the RIN-m rat islet cell line were purchased from the American Type Culture Collection (VA, USA). The cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Biowest, Nuaillé, France), streptomycin (100 μg/mL), and penicillin G (100 U/mL) (10% FBS/RPMI 1640) at 37°C in a 5% CO2 atmosphere. L6 myoblast cells were purchased from the European Collection of Animal Cell Culture (Wiltshire, UK) and cultured in Dulbecco's modified Eagle's medium (DMEM) with L-glutamine, streptomycin, penicillin G, and 10% FBS (10% FBS/DMEM) at 37°C in a humidified 5% CO2 incubator.

Assay of insulin secretion activity: The RIN-5F cells had been derived from rat pancreatic β-cells and were used to evaluate insulin secretion activity [20-22]. The cells were cultured on 24-well plates at a cell density of 4 × 105 cells/mL. After 72 h incubation, the medium in each well was replaced with 1 mL fresh medium, and the cells were incubated for a further 24 h. The medium was then removed from each well, and the cells were washed with fresh medium supplemented with 1% FBS.

Various concentrations of phytol (Wako, Osaka, Japan)or insulin (wako)were added to the wells, and the cells were incubated for 3h. An aliquot was then withdrawn from each well and centrifuged to separate the cells. The concentration of insulin in the medium was determined using an enzyme-linked immunosorbent assay (ELISA) (Morinaga Institute of Biological Science, Yokohama, Japan). The phytol was dissolved in dimethyl sulfoxide (DMSO) at a final concentration of 0.1% (v/v), and the insulin secretion level of samples with and without phytol were then compared.

Determination of glucose consumption by cultured L6 myotubes: A glucose consumption assay was performed on the L6 myotubes using the modified method of Doi et al. [23]. The L6 myoblasts (1 × 105 cells/mL)were maintained under subconfluent conditions in 24-well plates, and were cultured in 10% FBS/DMEM containing 5.5 mM glucose. The cells were differentiated over 8 days by reducing the serum concentration to 1% FBS containing either 5.5 mM glucose or 25 mM high glucose [20,24]. The myotubes were then maintained in filter-sterilized Krebs-Henseleit-Hepes buffer (KHH buffer; pH 7.4, 0.164 g/L KH2PO4, 0.192 g/L CaCl2 – 2H2O, 0.298 g/L MgSO4 – 7H2O, 0.36 g/L KCl, 2.38 g/L Hepes, 6.9 g/L NaCl, and 2.1 g/L NaHCO3) containing 0.1% bovine serum albumin, 2 mM pyruvate sodium, 5mM glucose, and 18mM mannitol for 2 h. The medium was then removed and replaced with 5 mMglucose and 17.5mM mannitol containing various concentration of phytol or insulin. The medium was collected at time zero and 4 h, and the glucose concentration was measured using the glucose oxidase method with the glucose CII-test (Wako). Glucose consumption was then calculated by subtracting the remaining glucose at the 4 h time point from that in the medium at time zero.

Immunoblot analysis: Using a similar method to the glucose consumption assay, L6 myoblasts (1 × 105 cells/mL) were seeded in 100 mm dishes and were maintained under subconfluent conditions in 10% FBS/DMEM containing 5.5 mM glucose. The cells were differentiated over8 days, following which the myotubes were placed in KHH buffer containing 0.1% bovine serum albumin, 2 mM sodium pyruvate, 5 mM glucose, and 18 mM mannitol for 2 h. The medium was then removed and replaced with 5 mM glucose, 17.5mM mannitol, and various concentration of phytol. After 3 h, the cells were collected and stored at– 80°C until further analysis.

The cells were rinsed with Ca2+/Mg2+ free phosphate-buffered saline,scraped from the 100mm dishes, and collected in a total volume of 100 μL of lysate buffer (50mM Tris (pH 8.0), 10mM NaF, 1mM Na3VO4, 0.5 mM 2-mercaptoethanol, 0.1% Triton X-100, 1% protease inhibitor cocktail (Calbiochem, CA, USA)) [25].The cells were then immediately homogenized. The protein concentration of the samples for immuoblotting was determined using the PierceTM BCA Protein Assay Kit (Thermo Scientific, MA, USA). Immunoblot detection was performed using Ez-Capture MG (ATTO, Tokyo, Japan). An anti-rat AKT antibody (#9272), anti-rat phosphorylated AKT antibody (#9271), anti-rat AMPK antibody (#2532),anti-rat phosphorylated AMPK antibody (#2531), and anti-rat β-actin (#4967) were obtained from Cell Signaling Technology (MA, USA). Anti-rabbit (W4011) IgG was purchased from Promega Corporation (WI, USA).

In vivo studies

Animals: Four-week-old male C57BL/6J mice were purchased from SLC Japan (Shizuoka, Japan)and fed a commercial CE-2 pellet diet (Clea Japan, Tokyo, Japan) for 7 days. The mice were then assigned to one of five experimental groups (n = 6), each of which contained mice of equal weights. The animals in four of these groups were fed a 45% high-fat diet (HFD) (D12451; Research Diets, NJ, USA), while the fifth group received a low-fat diet (LFD) (D12450B; Research Diets). The animals were placed in individual cages, maintained in a temperature-controlled room (23 ± 2°C) on a 12 h light/dark cycle, and water and food were provided ad libitum. The study was approved by the Institution Animal Care and Use Committee of the Tokyo University of Marine Science and Technology, Japan.

Oral glucose tolerance test: An oral glucose tolerance test (OGTT) was performed on mice fed HDF or LFD fed for 4 months [26]. The administration solutions were prepared with glucose solutions containing 0.5% carboxymethyl-cellulose and 0 mg/kg body weight (bw) phytol (HFD or LFD groups), or 12.5, 25, or 50 mg/kg bw phytol (HFD + phy 12.5, HFD + phy 25, or HFD + 50 mg/kg bw groups, respectively), and were orally administered at 2 g/kg body weight glucose following overnight fasting. Blood samples were collected from the tail vein before, and 15, 30, 60, and 90 min after the administration. Serum glucose levels were determined using the glucose CII-test (Wako), and serum insulin was measured using ELISA kits (Morinaga Institute of Biological Science). All kits were used in accordance with the manufacturer's protocols.

At the end of the treatment period, the mice were anesthetized and sacrificed with isoflurane (Wako) following overnight fasting. Subsequently, subcutaneous and visceral fat samples were collected, with visceral fat defined as the sum of the mesenteric, epididymal, and retroperitoneal fat pad weight.

Immunoblot analysis: Using a similar method to OGTT, each groups (LFD, HFD, HFD + phy 12.5, HFD + phy 25, and HFD + 50 mg/kg bw) were orally administered at 2 g/kg body weight glucose with/without phytol following overnight fasting. Then mice were anesthetized and sacrificed with isoflurane 15-20 min after the administration and skeletal muscle samples were obtained. The tissues were snap-frozen in liquid nitrogen and stored – 80°C until needed for further analysis.

The protein concentration of the samples for immunoblotting, which was homogenized with lysate buffer, was determined by using the Pierce TM BCA Protein Assay Kit.

Statistical analysis

Values were expressed as the means± standard errors (SE) of the mean. Differences between the treatment groups were identified using Dunnett's multiple comparison tests and were considered significant when p was < 0.05 and < 0.01.Calculations of areas under the curve (AUC) for plasma glucose and insulin responses were based on the trapezoid rule.

RESULTS AND DISCUSSION

In vitro, phytol stimulates insulin secretion and glucose uptake via activation of AKT and AMPK

To investigate the effect of phytol on insulin secretion, we examined insulin secretion by RIN-5F cells, a rat islet tumor cell line. Phytol significantly stimulated insulin secretion in a dose dependent manner (**Figure 1**).

In animals, phytol is converted to phytanic acid in several tissues [9,27]. The finding suggests that the insulin sensitizing/anti-diabetic effect of phytol is not only mediated by partly from activation of nuclear receptors and heterodimerization of RXR with PPARγ by phytanic acid [16], specifically in human skeletal muscle and adipocytes, but also act directly to pancreatic β-cells. Furthermore, because phytol is known to have antioxidantive effects [15], it may reduce oxidant stress in RIN-5F cells.

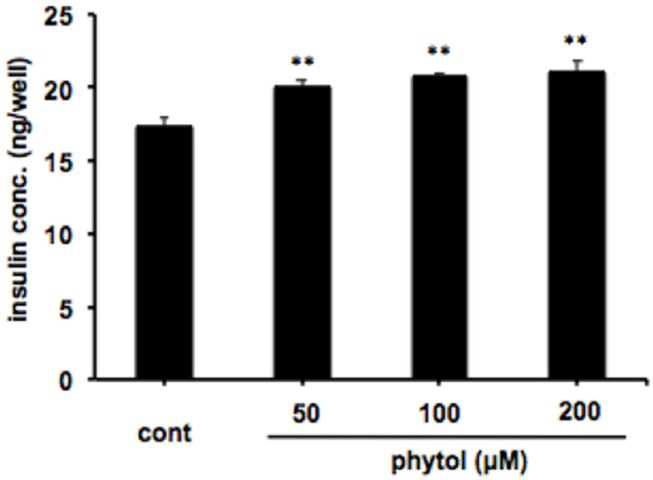

Figure 1. Effect of phytol on insulin secretion in cultured RIN-5F cells. Each value represents the mean ± SE (n = 6). **p < 0.01 *versus* control.

The effect of phytol on glucose uptake under normal glucose (5.5 mM) and high glucose (25mM) conditions was examined in L6 cells. These glucose conditions mimicked the normoglycemic condition and the hyperglycemic condition in diabetes, respectively [20,24]. Under normal glucose conditions, phytol concentrations of 50–200 μM significantly stimulated glucose uptake in a dose-independent manner (**Figure 2A**). In contrast, under high glucose conditions, maximum increase in glucose uptake was observed at 50 μM phytol (**Figure 2B).**

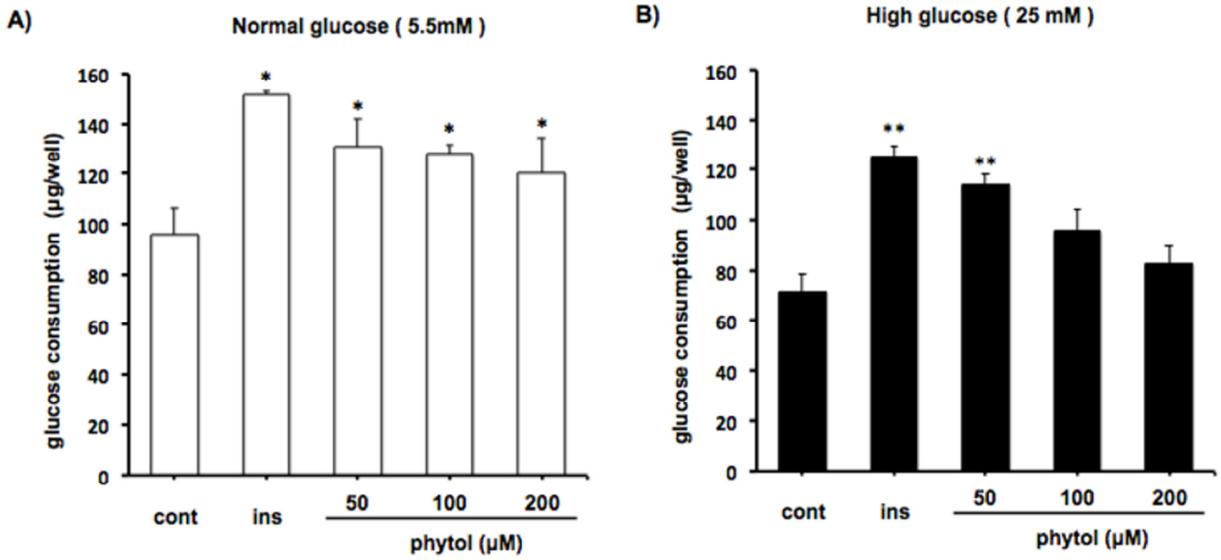

Figure 2. Effects of phytol on glucose uptake in cultured L6 myotubes under normal (A) and high (B) glucose conditions. L6 myotubes were pre incubated in KHH buffer without glucose for 2h. They were then incubated in KHH buffer containing 5 mM glucose and 50, 100, or 200 μM phytol for 4 h, and the glucose uptake was determined. Each value represents the mean ± SE (n = 6). *p < 0.05, **p < 0.01 *versus* control.

Therefore, it can be concluded that 50 μM is the optimal phytol concentration for normal glucose conditions, while < 50 μM is optimal for high glucose conditions. Importantly, these data showed that phytol had the same effect on glucose uptake in myotubes as observed with maximum insulin stimulation. A further investigation was performed to examine whether phytol increases glucose uptake in myoblasts with low GLUT4 expression, which are known not to respond to insulin [28]. In these L6 myoblasts, glucose uptake was not significantly increased by phytol, indicating that it may act by modulating GLUT4 translocation and/or activity (data not shown).

The effect of phytol on AKT phosphorylation was examined. Insulin stimulates the phosphorylation of both residues

associated with AKT activation (Thr308 and Ser473), but200 μM phytol also activated Ser473 in L6 cells (**Figure 3A**).A previous study showed that phytol strongly activates PPARα and also acts as a ligand, binding to this transcriptional factor [17]. PPARα expresses in skeletal muscle as well as heart and liver [29] and increases fatty acid oxidation by inducing AMPK activation [30]. Furthermore, several other phytochemicals have also been found to activate AMPK [31]. Therefore, we hypothesized that phytol would also activate AMPK in skeletal muscle. Supporting this, treatment of the cells with phytol resulted in a significant increase in AMPK-Thr172 phosphorylation, which is a known indicator of AMPK activation (**Figure 3B),** whereas AMPK was not activated by insulin. These phenomena by phytol were indicated in a dose-dependent manner. These results suggest that phytol enhance glucose uptake in the skeletal muscle by inducing GLUT4 translocation through activation of weak AKT and strong AMPK. Furthermore, activation of both AKT and AMPK in skeletal muscle by phytol may contribute to the insulin-sensitizing/anti-diabetic effect following its intake.

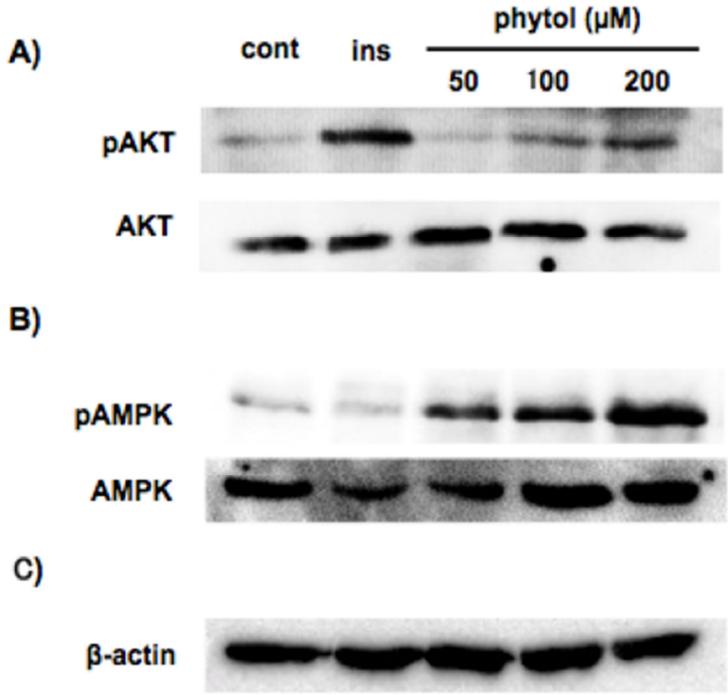

Figure 3. Effects of phytol on the phosphorylation of AKT (A) and AMPK (B). β-actin was used as a loading control (C). L6 myotubes were preincubated in KHH buffer without glucose for 2 h. They were then incubated in KHH buffer containing 5 mM glucose in the presence or absence of 50, 100, or 200 μM for 3 h. Total lysates were analyzed by immunoblotting.

Abnormal glucose metabolism in diet-induced obesity mice is improved by a single administration of phytol

Mice which were fed the HFD for four months became obese, exhibiting higher final body weights, and more subcutaneous and visceral fat than normal mice that received the LFD over the same period (**Table 1**).

Table 1. Final body weight, subcutaneous fat, and visceral fat in mice fed a low-fat diet (LFD) or a high-fat diet (HDF) for 4 months followed by the administration of 0, 12.5, 25, or 50 mg/kg body weight (bw) phytol (phy).

Group	Final body weight (g)	Subcutaneous fat (g)	Visceral fat (g)
LFD	34.3 ± 1.1	0.66 ± 0.05	2.43 ± 0.21
HFD	42.4 ± 0.9 **	1.63 ± 0.17 **	4.04 ± 0.05 **
HFD + phy 12.5 mg/kg bw	42.4 ± 2.0 **	1.46 ± 0.18 **	4.09 ± 0.22 **
HFD + phy 25 mg/kg bw	42.1 ± 1.6 **	1.64 ± 0.21 **	4.10 ± 0.17 **
HFD + phy 50 mg/kg bw	40.8 ± 2.0 *	1.59 ± 0.12 **	4.35 ± 0.14 **

Values are expressed as means ± SE (n = 6).
**, ** Means within a line are significantly different according to Dunnett's multiple comparison test, p < 0.05 or p < 0.01 versus LFD group, respectively.*

The OGTT showed that the administration of a glucose solution without phytol resulted in the serum glucose levels in the HFD group becoming significantly higher than those in the LFD group for 90 min after administration (**Figure 4A),** indicating that the diet-induced obese mice had abnormal glucose metabolism. In contrast, a single administration of a glucose solution containing 12.5 or 50 mg/kg bw phytol to obese mice significantly suppressed postprandial hyperglycemia compared with the HFD group. The serum glucose level in obese mice that were administered 25 mg/kg bw phytol was not significantly higher than the HFD group after 15 min. However, the AUC for serum glucose levels was significantly lower in all phytol groups compared with the HFD group (**Figue 4B**). This in vivo phenomenon may be similar to the earlier finding that phytol enhances glucose uptake in L6 myoblasts in vitro.

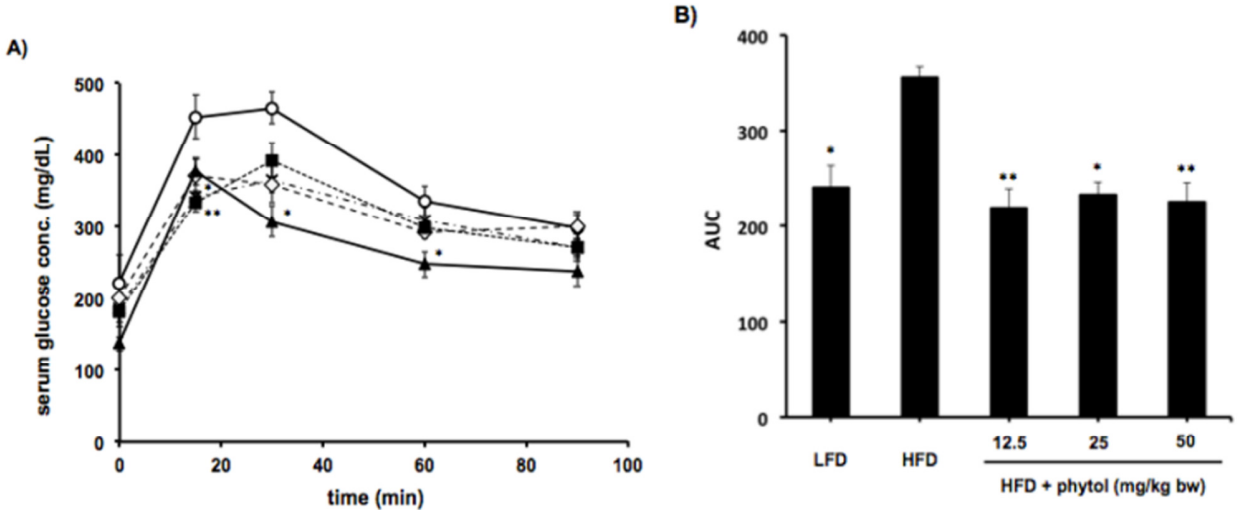

Figure 4. Serum glucose level (A) and area under the curve (B) during OGTT in obese mice administered glucose solutions containing 0, 12.5, 25, or 50 mg/kg bw phytol. OGTT was performed at 4 months period. For OGTT, glucose solutions (2 g glucose/kg bw) were orally administered after an overnight fasting. Each bar represents the mean ± SE (n = 6). (A) ▲, LFD (without phytol);○, HFD (without phytol); ✖, HFD + phy 12.5 mg/kg bw; ◇, HFD + phy 25 mg/kg bw; ■, HFD + phy 50 mg/kg bw; *$p < 0.05$, **$p < 0.01$ *versus* HFD group (without phytol). (B) *$p < 0.05$, **$p < 0.01$ *versus* HFD group.

Additionally, the obese mice that administrated 25 and 50 mg/kg phytol in a single administration tended to be suppressed rapidly increasing and reducing of serum insulin secretion from 15 min to 30 min after administration **(Figure 5A**). However, the AUC for serum insulin levels in these phytol groups was not significantly different, compared with that in HFD group (**Figure 5B**).Thus, a single administration of phytol improved the abnormal pattern of insulin secretion in obese mice without affecting the amount of insulin secretion. This in vivo phenomenon did not agree with the finding that phytol promotes insulin secretion in cultured RIN-5F cells in vitro. We hypothesize that phytol has different mechanisms acting on the pancreas depending on intake period. It has been reported that Asian patients with T2D are physiologically characterized by lower β-cell function and a lesser degree of insulin resistance, compared with their Caucasian counterparts [32]. A once-daily intake of phytol could contribute much to enhance β-cell function in T2D patients of the world, specifically many Asian countries. Moreover, the improvement mechanism of insulin secretion by phytol may be similar to the results of vitamin E, one of phytol metabolites [33].

We also examined that the effects of phytol on AKT and AMPK phosphorylation in skeletal muscle of obese mice. In mice administered phytol, only AMPK-Thr172 phosphorylation was drastically increased (**Figure 6A and B).** This in vivo phenomenon did not agree with the finding that phytol induced activation of AKT-Ser473.In order to clarify whether phytol induce directly AMPK activation in skeletal muscle and L6 cell, we demonstrated by using one of A MPK inhibitors, dolsomolphin, in L6 cell and found that co-adding dorsomolphin inhibited AMPK phosphorylation by phytol at 50–200μM (data not shown).It is possible that phytol activates strongly AMPK phosphorylation over AKT in vivo and enhances glucose uptake, especially in skeletal muscle.

High-fat diets have been shown to result in increased body weight and diabetes in various strains of mice and rats. Indeed, C57BL/6J mice are a particularly good model

mimicking human metabolic derangements that are observed in obesity because when fed ad libitum with a HFD, these mice develop obesity, hyperinsulinemia, hyperglycemia, and hypertension, but when fed ad libitum to chow diet, they remain lean without metabolic abnormalities [34]. The single administration of phytol to obese mice that were fed a HFD for four months suppressed the increases in postprandial blood glucose levels that occurred in the HFD group. Previous studies have shown that phytol is rapidly converted into phytanic acid in the tissues of mammals such as rats and humans [9,27]. However, Golerich et al. [35] reported that phytol accumulated in the liver of rats that were fed a phytol-enriched diet for 7 days. Thus, these in vivo results are likely to be caused by the administration of phytol.

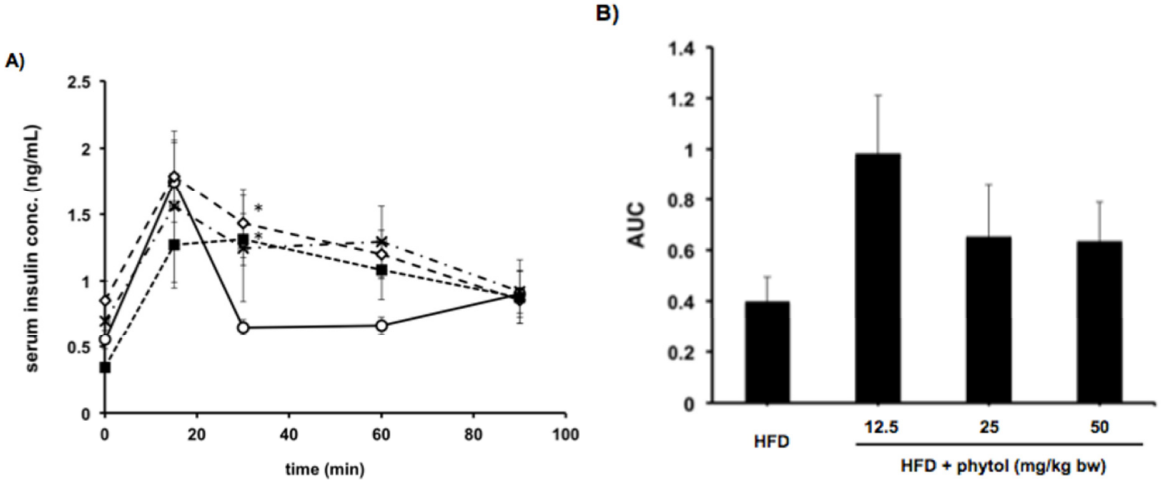

Figure 5. Serum insulin level (A) and areas under the curve (B) during OGTT in obese mice. Each bar represents the mean ± SE (n = 6). (A) ○, HFD (without phytol); ✖, HFD + phy 12.5 mg/kg bw; ◇, HFD + phy 25 mg/kg bw; ■, HFD + phy 50 mg/kg bw; *p < 0.05 *versus* HFD group (without phytol). (B) There is no difference compare with HFD group (without phytol).

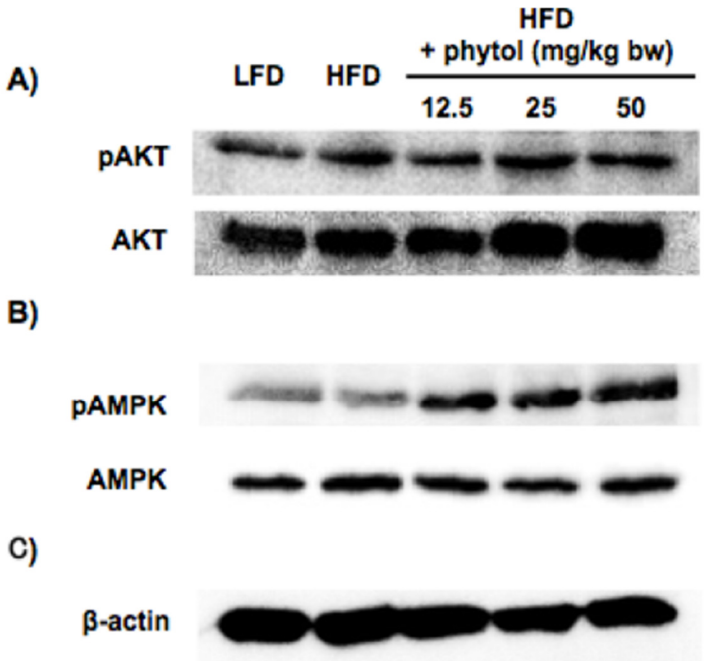

Figure 6. Effects of phytol on the phosphorylation of AKT (A) and AMPK (B) in skeletal muscle of obese mice 15 – 20 min after administration of glucose solutions containing 0, 12.5, 25, or 50 mg/kg bw phytol. β-actin was used as a loading control (C). The obtained skeletal muscle samples were homogenized with lysate buffer and total lysates were analyzed by immunoblotting.

CONCLUSIONS

The present study demonstrates, for the first time, that phytol improves the abnormal pattern of insulin secretion in pancreatic β-cells and directly stimulates muscle glucose uptake independent of insulin via AMPK activation in vivo. Moreover, a single administration of phytol improved glucose tolerance in obese mice that were exhibiting abnormal insulin secretion. Besides previous studies on the effects of the long-term intake of phytol in rodents, these data confirmed the glucose uptake mechanism by intake of dietary phytol and the immediate effects of phytol's health promoting ability.

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REFERENCES

1. Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, et al. (2001) Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. The N Engl J Med 345: 790-797.
2. DeFronzo RA, Tripathy D (2009) Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. Diabetes Care 32: 157-163.
3. Stanford KI, Goodyear LJ (2014) Exercise and type 2 diabetes: molecular mechanisms regulating glucose uptake in skeletal muscle. Advan Physiol Educat 38: 308-314.
4. Saltiel AR, Kahn CR (2001) Insulin signaling and the regulation of glucose and lipid metabolism. Nature 414: 799-806.
5. Towler MC, Hardie DG (2007) AMP-activated protein kinase in metabolic control and insulin signaling. Circ Res 10: 328-341.
6. Fryer LG, Parbu-Patel A, Carling D (2002) The Anti-diabetic drugs rosiglita zone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. J Biol Chem 277: 25226-25232.
7. Musi N, Goodyear LJ (2003) AMP-activated protein kinase and muscle glucose uptake. Acta Physiol Scand 178: 337-345.
8. Swiezewska E, Danikiewicz W (2005) Polyisoprenoids: structure, biosynthesis and function. Prog Lipid Res 44: 235-258.
9. van den Brink DM, Wanders RJ (2006) Phytanic acid: production from phytol, its breakdown and role in human disease. Cell Mol Life Sci 63: 1752-1765.
10. Valentin HE, Lincoln K, Moshiri F, Jensen PK, Qi Q, et al. (2006) The Arabidopsis vitamin E pathway gene5-1 mutant reveals a critical role for phytol kinase in seed tocopherol biosynthesis. Plant cell 18: 212-224.
11. Lampe JW, Chang JL (2007) Interindividual differences in phytochemical metabolism and disposition. Semin Cancer Biol 17: 347-353.
12. Baxter JH (1968) Absorption of chlorophyll phytol in normal man and in patients with Refsum's disease. J Lipid Res 9: 636-641.
13. Hu X, Makita S, Schelbert S, Sano S, Ochiai M, et al. (2015) Reexamination of chlorophyllase function implies its involvement in defense against chewing herbivores. Plant Physiol 167: 660-670.
14. Lee KI, Rhee SH, Park KY (1999) Anticancer Activity of Phytol and Eicosatrienoic Acid Identified from Perilla Leaves. J Kor Soc Food Sci Nutr 28: 1107-1112.
15. Santos CC, Salvadori MS, Mota VG, Costa LM, de Almeida AA, et al. (2013) Antinociceptive and Antioxidant Activities of Phytol In Vivo and In Vitro Models. Neurosci J.
16. Elmazar MM, El-Abhar HS, Schaalan MF, Farag NA (2013) Phytol/Phytanic acid and insulin resistance: potential role of phytanic acid proven by docking simulation and modulation of biochemical alterations. PLoS ONE 8: e45638.
17. Goto T, Takahashi N, Kato S, Egawa K, Ebisu S, et al. (2005) Phytol directly activates peroxisome proliferator-activated receptor α (PPARα) and regulates gene expression involved in lipid metabolism in PPARα-expressing HepG2 hepatocytes. Biochem Biophys Res Commun 337: 440-445.
18. Matsuda H, Gomi RT, Hirai S, Egashira Y (2013) Effect of dietary phytol on the expression of α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase, a key enzyme of tryptophan-niacin metabolism, in rats. Biosci Biotechnol Biochem 77: 1416-1419.
19. Yoshino J, Mills KF, Yoon MJ, Imai S (2011) Nicotinamide mononucleotide, a key NAD(+) intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. Cell Metab 14: 528-536.
20. Ha BG, Nagaoka M, Yonezawa T, Tanabe R, Woo JT, et al. (2012) Regulatory mechanism for the stimulatory action of genistein on glucose uptake in vitro and in vivo. J Nutr Biochem 23: 501-509.
21. Mujića A, Grdovićb N, Mujićc I, Mihailovićb M, Živkovićd J, et al. (2011) Antioxidative effects of phenolic extracts from chestnut leaves, catkins and

spiny burs in streptozotocin-treated rat pancreatic β-cells. Food Chem. 125: 841-849.

22. Nomura E, Kashiwada A, Hosoda A, Nakamura K, Morishita H, et al. (2003) Synthesis of amide compounds of ferulic acid, and their stimulatory effects on insulin secretion in vitro. Bioorg Med Chem 11: 3807-3813.
23. Doi M, Yamaoka I, Fukunaga T, Nakayama M (2003) Isoleucine, a potent plasma glucose-lowering amino acid, stimulates glucose uptake in C2C12 myotubes. Biochem Biophys Res Commun 312: 1111-1117.
24. Zhang W, Liu J, Tian L, Liu Q, Fu Y, Garvey WT (2013) TRIB3 mediates glucose-induced insulin resistance via a mechanism that requires the hexosamine biosynthetic pathway. Diabetes 62: 4192-4200.
25. Nishiumi S, Ashida H (2007) Rapid preparation of a plasma membrane fraction from adipocytes and muscle cells: application to detection of translocated glucose transporter 4 on the plasma membrane. Biosci Biotechnol Biochem 71: 2343-2346.
26. Kim YI, Hirai S, Goto T, Ohyane C, Takahashi H, et al. (2012) Potent PPARα activator derived from tomato juice, 13-oxo–9,11-octadecadienoic acid, decreases plasma and hepatic triglyceride in obese diabetic mice. PLoS ONE 7: e31317.
27. Steinberg D (1996) The Metabolic and Molecular Bases of Inherited Disease. (7th edn), McGraw-Hill, NY.
28. Mitsumoto Y, Burdett E, Grant A, Klip A (1991) Differential expression of the GLUT1 and GLUT4 glucose transporters during differentiation of L6 muscle cells. Biochem Biophys Res Commun. 175: 652-659.
29. Burri L, Thoresen GH, Berge RK (2010) The Role of PPARα Activation in Liver and Muscle. PPAR Res 1-11.
30. Lee WJ, Kim M, Park HS, Kim HS, Jeon MJ, et al. (2006) AMPK activation increases fatty acid oxidation in skeletal muscle by activating PPARα and PGC-1. Biochem Biophys Res Commun 340: 291295.
31. Lee CG, Koo JH, Kim SG (2015) Phytochemical regulation of Fyn and AMPK signaling circuitry. Arch Pharm Res 38: 2093-2105.
32. Kim YG, Hahn S, Oh TJ, Park KS, Cho YM (2014) Differences in the HbA1c-lowering efficacy of glucagon-like peptide-1 analogues between Asians and non-Asians: a systematic review and meta-analysis. Diabetes Obes Metab 16: 900-909.
33. Ihara Y, Yamada Y, Toyokuni S, Miyawaki K, Ban N, et al. (2000) Antioxidant α-tocopherol ameliorates glycemic control of GK rats, a model of type 2 diabetes. FEBS Lett 473: 24-26.
34. Collins S, Martin TL, Surwit RS, Robidoux J (2004) Genetic vulnerability to diet-induced obesity in the C57BL/6J mouse: physiological and molecular characteristics. Physiol Behav 81: 243-248.
35. Gloerich J, van Vlies N, Jansen GA, Denis S, Ruiter JP, et al. (2005) A phytol-enriched diet induces changes in fatty acid metabolism in mice both via PPARalpha-dependent and -independent pathways. J Lipid Res 46: 716-726.