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Phytic acid and *myo*-inositol support adipocyte differentiation and improve insulin sensitivity in 3T3-L1 cells

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ABSTRACT

Phytic acid, also known as *myo*-inositol hexaphosphate, has been shown to lower blood glucose levels and to improve insulin sensitivity in rodents. We investigated the effects of phytic acid and *myo*-inositol on differentiation, insulin-stimulated glucose uptake, and lipolysis of adipocytes to test the hypothesis that the antidiabetic properties of phytic acid and *myo*-inositol are mediated directly through adipocytes. 3T3-L1 cells were treated with 10, 50, or 200 μmol/L of phytic acid or *myo*-inositol. Oil Red O staining and an intracellular triacylglycerol assay were used to determine lipid accumulation during adipocyte differentiation. Immunoblotting and real-time polymerase chain reaction (PCR) were performed to evaluate expression of transcription factors, a target protein, and insulin signaling molecules. Phytic acid and *myo*-inositol exposures increased lipid accumulation in a dose-dependent manner ($P < .01$). The expression of key transcription factors associated with adipocyte differentiation, such as peroxisome proliferator-activated receptor γ (PPARγ) and sterol regulatory element-binding protein 1c, and the expression of fatty acid synthase increased upon treatments with phytic acid and *myo*-inositol ($P < .05$). Insulin-stimulated glucose uptake in mature adipocytes increased with phytic acid and *myo*-inositol treatments ($P < .01$). In addition, mRNA levels of insulin receptor substrate 1 (IRS1), mRNA levels of glucose transporter 4, and phosphorylation of tyrosine in IRS1 increased upon phytic acid and *myo*-inositol treatments. In fully differentiated adipocytes, phytic acid and *myo*-inositol reduced basal lipolysis dose dependently ($P < .01$). These results suggest that phytic acid and *myo*-inositol increase insulin sensitivity in adipocytes by increasing lipid storage capacity, improving glucose uptake, and inhibiting lipolysis.

Abbreviations: 2-NBDG, 2-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose; ANOVA, analysis of variance; C/EBP, CCAAT/enhancer-binding protein; DMEM, Dulbecco's modified Eagle's medium; FAS, fatty acid synthase; FBS, fetal bovine serum; GLUT, glucose transporter; IRS, insulin receptor substrate; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PPAR, peroxisome proliferator-activated receptor; SREBP, sterol regulatory element-binding protein; TG, triacylglycerol; BCS, bovine calf serum; PI3K, phosphoinositide 3-kinase; PKB, protein kinase B.

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1. Introduction

Type 2 diabetes mellitus is a metabolic disorder characterized by high blood glucose levels and insulin resistance in peripheral tissues, such as skeletal muscle, adipose tissue, and liver tissue. Because chronic hyperglycemia causes complications, such as cardiovascular disease, renal failure, and retinopathy [1], the goals of type 2 diabetes mellitus therapies are to control blood glucose levels and to improve insulin sensitivity.

It is generally recommended that diabetic patients consume whole grains and cereals to control their blood glucose levels [2]. Phytic acid, also known as *myo*-inositol hexaphosphate, is abundant in legumes, cereals, nuts, and whole grains in concentrations of 0.4% to 6.4% (by weight) [3]. Although it has been considered an antinutrient because it forms insoluble complexes with minerals and inhibits mineral absorption [4], numerous studies have shown that phytic acid is beneficial and has antioxidant, hypolipidemic, and anticarcinogenic activities [5–7]. In addition, it has been reported that phytic acid lowers blood glucose levels and improves glucose tolerance in normal and diabetic rodents [8,9]. In healthy volunteers, blood glucose responses (glycemic indices) were negatively correlated with the phytic acid content of the food consumed [10]. Although the mechanisms underlying this association are not yet fully understood, it has been proposed that inhibition of α-amylase activity and starch digestion by phytic acid correlates with an attenuated rise in postprandial blood glucose levels [11,12]. Considering the ability and importance of insulin to mediate glucose responses in metabolically active target cells, to maintain glucose homeostasis, and to clear postprandial glucose loads, this proposal provides a partial explanation [13]. Phytic acid is an abundant inositol phosphate in cells and can be produced from inositol or its form with fewer phosphate groups by metabolic conversion [14]. *Myo*-inositol, the most common isomer of inositol in tissues and in food, has been shown to lower plasma glucose levels by triggering glucose transporter 4 (GLUT4) translocation and to improve insulin sensitivity by increasing protein kinase B (PKB)/Akt phosphorylation in the skeletal muscle of mice [15,16].

In addition to skeletal muscle, adipocyte responses to insulin should be investigated because adipose tissue is one of the major target tissues of insulin and because disruptions in glucose uptake in adipose tissue have been found to lead to insulin resistance in mice [17]. Adipocyte differentiation requires the synergistic actions of multiple transcription factors, primarily CCAAT/enhancer-binding protein α (C/EBP α), peroxisome proliferator-activated receptor γ (PPARγ), and sterol regulatory element-binding protein 1c (SREBP1c) [18,19]. PPARγ, the master regulator of adipocyte differentiation, activates glucose metabolism and in turn increases insulin sensitivity [20,21]; thus, PPARγ is a target for antidiabetic drugs, such as thiazolidinediones [22]. In addition, it has been reported that an increase in the release of free fatty acids from stored fat attenuates insulin sensitivity [23]. Nevertheless, few studies have investigated the effects of phytic acid and *myo*-inositol on adipocytes, and the cellular mechanisms underlying their antidiabetic properties are unknown.

We hypothesized that phytic acid and *myo*-inositol increase adipocyte differentiation and insulin sensitivity. To examine the effects of phytic acid and *myo*-inositol on adipocyte differentiation, we measured lipid accumulation and expression of adipogenic transcription factors during differentiation of murine preadipocyte 3T3-L1 cells. To investigate insulin sensitivity, we assayed for lipolysis of mature adipocytes, insulin-stimulated glucose uptake, and expression of insulin-signaling proteins in adipocytes. The well-characterized murine preadipocyte 3T3-L1 cells were used to test the direct effect of phytic acid and *myo*-inositol. By determining the cellular mechanisms that contribute to the antidiabetic properties of phytic acid and *myo*-inositol, we can further understand the benefits of a whole grain diet.

2. Methods and materials

2.1. Materials

Phytic acid (dodecasodium salt from rice, # P0109) and *myo*-inositol were purchased from Sigma Aldrich (St. Louis, MO, USA). Bovine calf serum (BCS), fetal bovine serum (FBS), penicillin-streptomycin-glutamine, Dulbecco's modified Eagle's medium (DMEM), and 2-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose (2-NBDG) were purchased from Invitrogen (Carlsbad, CA, USA). All additional chemicals were obtained from Sigma Aldrich.

2.2. Cell culture

3T3-L1 mouse embryo fibroblasts were obtained from the American Type Culture Collection (Manassas, VA, USA) and cultured as described by Hemati et al [24]. Briefly, cells were cultured in DMEM containing 10% bovine calf serum until they reached confluency. To induce differentiation, 2-day postconfluent 3T3-L1 preadipocytes (D0) were stimulated with 0.5 μmol/L of isobutylmethylxanthine (IBMX), 1 μmol/L of dexamethasone, and 167 nmol/L of insulin in DMEM containing 10% FBS (MDI differentiation medium) for 2 days (D2). Cells were maintained in 10% FBS/DMEM with 167 nmol/L of insulin for 2 more days (D4) and then were cultured in 10% FBS/DMEM for an additional 4 days (D8), at which time more than 90% of cells were mature adipocytes filled with lipid droplets. All media contained penicillin (100 U/mL), streptomycin (100 μg/mL), and glutamine (292 μg/L). Cultures were maintained at 37°C under humidified 5% CO_2. Phytic acid and *myo*-inositol were dissolved in phosphate-buffered saline (PBS) before addition to cell cultures. Phosphate-buffered saline was used as the control.

2.3. MTS assay

The MTS assay [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt, MTS; CellTiter 96 AQueous One Solution, Promega, Madison, WI, USA] was performed to determine the number of viable cells per culture. The assay was performed, according to the manufacturer's instructions, when cells were in the preconfluent exponential growth phase and when cells