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Monoterpenes: Novel insights into their biological effects and roles on glucose uptake and lipid metabolism in 3T3-L1 adipocytes

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ABSTRACT

Various strategies have been adopted to combat complications caused by Type 2 diabetes mellitus and controlled diet is one of them. Monoterpenes, major constituents of essential oils, are synthesized and widely used as artificial food flavors. A series of twelve monoterpenes were assessed in the present study. Monoterpenes, exhibited low 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) and 2,2′-azinobis-(3-ethyl benzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity even at high concentrations. Some monoterpenes inhibited α-amylase and α-glucosidase activity and stimulated glucose uptake and lipolysis. Monoterpenes such as (R)-(+)-limonene stimulated both glucose uptake (17.4%) and lipolysis (17.7%); the mRNA expression of glucose transporter 1 (GLUT1) was upregulated but glucose transporter 4 (GLUT4) was unaffected, and adipose triglyceride lipase (ATGL) was suppressed. Taken together, the selected monoterpenes may not confer strong protection against free radicals but nevertheless, their positive influence on lipid and glucose metabolism may have potential in the control of obesity and Type 2 diabetes mellitus.

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1. Introduction

Monoterpenes are amongst the major constituents of essential oils and abundantly found in citrus fruits, vegetables, spice and herbs. Monoterpenes are a class of terpenes, consisting of two isoprene units in the structures, which can either be in the form of acyclic (linear) or cyclic (ring). Monoterpenes are volatile compounds and characterized by strong odors responsible for the fragrance in many plants and fruits. Monoterpenes which are well known for their fragrance have been commercially used as artificial flavors in food, sanitary, cosmetic and perfume industries. In the past decades, increasing evidence showing biological effects of monoterpenes, such as antioxidant, anti-phlogistic, anti-tumor, antiviral and anti-nociceptive properties have intensified the usage and consumption of monoterpenes (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Choi, Song, Ukeda, & Sawamura, 2000; Kamatou, Vermaak, Viljoen, & Lawrence, 2013). Dietary intake of monoterpenes in human is fairly high. For instance, concentration of d-limonene, a fragrance additive with a lemon-like odor in candy, ice cream, orange juice and chewing gum is 49 ppm, 68 ppm, 100 ppm, and 2300 ppm, respectively. The U.S. per capita dietary consumption of both naturally occurring and artificial d-limonene is 0.27 mg/kg body weight/day. By this large scale consumption of monoterpenes, significant beneficial biological effects could possibly be reflected on human health in the long run. Evidence from the phase I clinical trials had showed that oral consumption of d-limonene in advanced cancer patients helped in the control of breast cancer and colorectal carcinoma which further strengthens the benefits of dietary monoterpenes in human health (Sun, 2007).

There have been numerous studies focused primarily on anti-cancer and anxiolytic properties of monoterpenes but not much is known with regards to their effects on obesity and Type 2 diabetes mellitus (T2DM). T2DM is one of the metabolic disorders characterized by chronic hyperglycemia due to impaired insulin production, insulin function, or both. T2DM is associated with complications such as retinopathy, nephropathy, peripheral neuropathy, cardiovascular complications and sexual dysfunction. Generally, obesity is the main predisposing factor to T2DM due to the increased insulin resistance in obese people (American Diabetes Association, 2014). Several effective interventions have been implemented over the past decades for the effective control of diabetes, such as changes in diet and exercise. In recent years, there is emerging evidence which indicates that quality and type of food are highly associated with the risk factors of T2DM. For instance, whole-grain diets with high fiber and polyunsaturated fat are associated with a lower risk of T2DM whereas diets with

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high *trans* fat increase the risk of T2DM (Hu, 2011). More interestingly, diet can induce changes in the composition of gut microbiota which then alters the metabolism of short-chain fatty acids (SCFA). SCFA such as butyrate have been shown to improve insulin resistance in dietary-obese mice (Shen, Obin, & Zhao, 2013). Collectively, these findings suggest that components in diet play important roles in the development of T2DM. This necessitates further studies and better understanding on food components.

Therefore, in the current study, the biological effects of monoterpenes were studied and their roles on glucose uptake and lipid metabolism in 3T3-L1 adipocytes were investigated. 3T3-L1 adipocytes were used as they are one of the most common cell culture models used in the study of obesity and T2DM due to their roles on glucose uptake, lipogenesis and lipolysis which contribute directly to the control of energy balance (Arsenijevic, Gregoire, Delforge, Delporte, & Perret, 2012). By understanding the biological effects of food components including additives such as monoterpenes, diet can be more effectively planned for a better control of obesity and T2DM.

2. Materials and methods

2.1. Materials

Geraniol (PubChem CID: 637566), nerol (PubChem CID: 643820), citral (PubChem CID: 638011), (R)-(−)-linalool (PubChem CID: 443158), (R)-(+)-limonene (PubChem CID: 440917), (S)-(−)-perillyl alcohol (PubChem CID: 369312), (R)-(+)-β-citronellol (PubChem CID: 101977), (S)-(−)-β-citronellol (PubChem CID: 7793), α-terpineol (PubChem CID: 443162), l-menthol (PubChem CID: 16666), γ-terpinene (PubChem CID: 7461) and terpinolene (PubChem CID: 11463) were purchased from Sigma–Aldrich (USA).

2.2. 2,2-Diphenyl-2-picrylhydrazyl hydrate (DPPH) radical scavenging activity

DPPH radical scavenging activity of monoterpenes was assessed as described by Tan et al. (2015) with slight modifications. To examine the DPPH radical scavenging activity of monoterpenes, 10 μl of test sample was mixed with 290 μl of 0.1 mM of DPPH ethanol solution and incubated for 3 h in the dark at room temperature prior to the measurement of absorbance at 517 nm. Ascorbic acid was used as a positive control. The radical-scavenging activity of test sample was expressed in percentage of inhibition, based on the following equation, DPPH radical scavenging activity (%) = (OD of blank − OD of test compound)/OD of blank × 100.

2.3. 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity

ABTS radical scavenging activity of monoterpenes was assessed as described by Tan et al. (2015) with slight modifications. ABTS radical monocations were generated by mixing 5 ml of 7 mM ABTS solution and 89 μl of 2.45 mM potassium persulphate ($K_2O_8S_2$). The solution was incubated for 12–16 h at room temperature in the dark before use. The working solution was prepared by diluting the previous solution with ethanol to obtain an absorbance of 0.7 ± 0.02 at a wavelength of 734 nm. The absorbance was measured using a spectrophotometer (Bio-Tech Instruments Inc, USA). Next, 10 μl of the test sample was added to 90 μl of the working solution in a 96-well plate and incubated for 2 h before the absorbance was measured. Trolox was used as the positive control. The radical-scavenging activity of the test sample was expressed in percentage of inhibition, calculated according to the following equation, ABTS radical scavenging activity (%) = (OD of blank − OD of test compound)/OD of blank × 100.

2.4. Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power of monoterpenes was evaluated as described by Tan et al. (2015) with slight modifications. FRAP reagent was prepared by mixing 300 mM acetate buffer (pH of 3.6), 10 mM 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) in 40 mM hydrochloric acid and 20 mM ferric chloride ($FeCl_3{\cdot}6H_2O$) in a ratio of 10:1:1. The resulting orange-coloured reagent was incubated at 37 °C before use. To evaluate the reducing power of test sample, 10 μl of test sample was added to 290 μl of FRAP reagent for 3 h. The absorbance of the mixture was measured at 593 nm. Ferrous sulphate ($FeSO_4{\cdot}7H_2O$) was used as a positive control. The results were expressed as FRAP value (mol $FeSO_4{\cdot}7H_2O$/mol test compound).

2.5. α-Glucosidase inhibitory activity

α-Glucosidase solution was prepared by dissolving α-glucosidase from Saccharomyces *cerevisiae* in 0.1 M sodium phosphate buffer pH 6.8 supplemented with 0.2% bovine serum albumin (BSA) to give a concentration of 0.4 U/mL. Then, equal volumes (20 μl) of test sample, 0.4 U/mL α-glucosidase solution and 6 mM 4-nitrophenyl-α-d-glucopyranoside (PNPG) were mixed and allowed to stand at 37 °C for 15 min. To terminate the reaction, 80 μl of 0.2 M sodium carbonate was added to the reaction mixture and the absorbance was measured at 400 nm. Acarbose was used as a positive control. The α-glucosidase inhibitory activity of monoterpenes was expressed in percentage of inhibition, calculated as follows, α-Glucosidase inhibitory activity (%) = (OD of control − OD of test sample)/OD of control × 100 (Manaharan et al., 2011).

2.6. α-Amylase inhibitory activity

α-Amylase solution (2 U/ml) was prepared by dissolving porcine pancreatic α-amylase (Sigma Type IV-B) in ice-cold distilled water. Next, potato soluble starch solution (1%) was prepared by boiling starch in 20 mM phosphate buffer pH 6.9 until the solution became fully transparent. Then, 80 μl of the test sample was added to 40 μl of α-amylase solution. After 10 min incubation at room temperature, 40 μl of starch solution was added to the reaction mixture and allowed to stand for 10 min at 37 °C. To terminate the reaction, 80 μl of 3,5-dinitrosalicylic acid (DNS) solution consisting of 1 g DNS and 30 g sodium potassium tartrate dehydrate (dissolved in 100 ml of 2 M sodium hydroxide) was added and incubated at 95 °C for 10 min. The absorbance was measured at 540 nm. Acarbose was used as a positive control. The α-amylase inhibitory activity of monoterpenes was expressed in percentage of inhibition, calculated as follows, α-Amylase inhibitory activity (%) = (OD of control − OD of test sample)/OD of control × 100 (Manaharan et al., 2011).

2.7. 3T3-L1 cell culture and differentiation

Murine 3T3-L1 pre-adipocytes were grown and induced to differentiate into mature adipocytes as described by Kanagasabapathy, Chua, Malek, Vikineswary, and Kuppusamy (2014) with slight modifications. 3T3-L1 pre-adipocytes (American Type Culture Collection, Rockville, MD, USA) were grown in Dulbecco's Modified Eagle's Medium (DMEM) with 10% (v/v) fetal bovine serum (FBS), 1% (v/v) penicillin–streptomycin (10,000 U/mL penicillin and 10,000 μg/mL streptomycin) and 4 mM L-glutamine at 37 °C in 95% air and 5% CO_2. To induce adipocyte

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