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Bitter melon triterpenes work as insulin sensitizers and insulin substitutes in insulin-resistant cells

Chi-I Chang [a], *Chang-Hung Chou* [b,*], *Ming-Huei Liao* [c], *Tz-Min Chen* [a], *Chia-Hsin Cheng* [a], *Rista Anggriani* [a,d], *Chung-Pao Tsai* [a], *Hsin-I Tseng* [a], *Hsueh-Ling Cheng* [a,**]

[a] *Department of Biological Science and Technology, National Pingtung University of Science and Technology, No. 1, Shuehfu Rd., Neipu, Pingtung 91201, Taiwan*

[b] *Research Center for Biodiversity, China Medical University, 91, Hsueh-Shih Road, Taichung, 40402, Taiwan*

[c] *Department of Veterinary Medicine, National Pingtung University of Science and Technology, No. 1, Shuehfu Rd., Neipu, Pingtung 91201, Taiwan*

[d] *Department of Agricultural Product Technology, Brawijaya University, Jalan Veteran Malang 65145, Indonesia*

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ABSTRACT

The triterpenes 3β,25-dihydroxy-7β-methoxycucurbita-5,23(E)-diene (DHM) and 3β,7β,25-trihydroxycucurbita-5,23(E)-dien-19-al (THC) were previously isolated from *Momordica charantia* (bitter melon) and identified as hypoglycaemic principles. This study further investigated their hypoglycaemic mechanisms. FL83B cells were treated with tumour necrosis factor-α to result in insulin resistance, a feature of type 2 diabetes. DHM and THC increased the tyrosine phosphorylation of insulin receptor substrate isoform 1 and the phosphorylation of Akt only in the presence of insulin in insulin-resistant cells, suggesting that they are insulin sensitizers. However, they enhanced the phosphorylation of AS160 (Akt substrate of 160 kDa), the migration of glucose transporter-4 and the glucose uptake of insulin-resistant cells in the absence of insulin, suggesting that they can substitute for insulin to promote glucose clearance. The insulin substitution function was blocked by an AMP-activated protein kinase (AMPK) inhibitor, whereas the insulin-sensitizing function may involve the inhibition of protein-tyrosine phosphatase-1B (PTP-1B). The IC_{50} of DHM and THC to PTP-1B is 92.84 μM and 25.42 μM, respectively. In summary, DHM and THC have insulin-sensitizing and insulin-substitution functions, which are likely correlated with their effects on inhibiting PTP-1B and activating AMPK, respectively.

* *Corresponding author*. Research Center for Biodiversity, China Medical University, 91, Hsueh-Shih Road, Taichung, 40402, Taiwan. Tel.: +886 4 2205366 ext 1633; fax: +886 4 22071500.
E-mail address: choumasa@mail.cmu.edu.tw (C-H. Chou).
** *Corresponding author*. Department of Biological Science and Technology, National Pingtung University of Science and Technology, No. 1, Shuehfu Rd., Neipu, Pingtung 91201, Taiwan. Tel.: +886 8 7703202 ext 5186; fax: +886 8 774 0550.
E-mail address: hlcheng@mail.npust.edu.tw (H-L. Cheng).

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1. Introduction

Diabetes mellitus is one of the most prevalent chronic disorders worldwide, and type 2 diabetes accounts for over 90% of diabetic cases. Type 2 diabetes is featured by insulin resistance, which occurs when the insulin-responsive tissues, mainly skeletal muscle, adipose tissue, and the liver, cannot respond to insulin properly (Hirabara et al., 2012; Tang, Li, Liu, Huang, & Ho, 2013). Insulin promotes the glucose uptake of insulin-sensitive tissues and inhibits glucose export from the liver; thus, insulin resistance causes hyperglycaemia and stimulates pancreatic β cells to secrete more insulin for maintaining a normal level of blood glucose, which will result in β-cell damage and the development of type 2 diabetes without prompt treatment (Sidor & Gramza-Michałowska, in press). Insulin resistance is also correlated with several other chronic diseases, such as dyslipidaemias, cardiovascular diseases, neurodegenerative disorders, and cancers (Hirabara et al., 2012). Agents or food supplements that can treat insulin resistance or provoke insulin-independent glucose disposal should be helpful in preventing β-cell damage, and managing type 2 diabetes and related disorders.

The precise mechanism of insulin resistance is not yet fully understood, whereas how obesity leads to the development of insulin resistance has been extensively investigated. When insulin binds to the cell-surface insulin receptor, the tyrosine-kinase activity of the receptor is activated, and several intracellular docking proteins are recruited to the receptor, among which insulin receptor substrates (IRSs), mainly IRS-1 and IRS-2, are closely related to glucose metabolism. The receptor catalyzes the tyrosine phosphorylation of the IRSs, resulting in the activation of PI3K (phosphatidylinositol-3-kinase), PDK1 (PI3K-dependent kinase), and subsequently Akt (protein kinase B). Akt catalyzes the phosphorylation of AS160 (Akt substrate of 160 kDa), which causes the migration of glucose transporter-4 (GLUT-4) from its cytoplasmic pool to the cell membrane. Cell-surface GLUT-4 facilitates the uptake of extracellular glucose by the cell, resulting in the reduction of blood glucose (Saltiel & Kahn, 2001; Whitehead, Clark, Urso, & James, 2000). In obese individuals, an overaccumulation of lipids in adipose tissue has been suggested to result in the production of proinflammatory cytokines such as tumour necrosis factor-α (TNF-α) and interleukin-1β from the adipocytes and the infiltrating leukocytes. The circulating cytokines cause low-grade chronic inflammation throughout the body and interfere with the insulin-signalling pathway in cells, resulting in insulin resistance (Baboota et al., 2013; Kolb & Mandrup-Poulsen, 2010). TNF-α inhibits insulin signalling through several mechanisms, including the promotion in the expression of protein-tyrosine phosphatase-1B (PTP-1B). PTP-1B removes the phosphorylation on tyrosine residues of the insulin receptor and the IRSs, resulting in the down-regulation of insulin signalling (Nieto-Vazquez et al., 2007). Therefore, in previous studies, we used TNF-α to induce insulin resistance in FL83B liver cells (Chang et al., 2011, 2014). The insulin-stimulated glucose uptake and IRS-1 tyrosyl phosphorylation of the cells were obviously suppressed by TNF-α treatment. To explore the hypoglycaemic constituents in antidiabetic medicinal plants such as *Momordica charantia*, we used such cells as a model to screen for natural products that promoted the glucose uptake of insulin-resistant cells (Chang et al., 2011; Cheng, Huang, Chang, Tsai, & Chou, 2008).

M. charantia L., belonging to the Cucurbitaceae family, is also known as bitter gourd or bitter melon, and has been used as an herbal medicine to treat hyperglycaemia in India, South America, and Asia (Grover & Yadav, 2004; Xie & Du, 2011; Zhang et al., 2014). Various extracts and some ingredients of the bitter melon have been reported in scientific articles to have hypoglycaemic functions in animal models of type 1 or type 2 diabetes (Chang et al., 2011; Harinantenaina et al., 2006; Nerurkar, Lee, Motosue, Adeli, & Nerurkar, 2008). Several types of medicinal compounds have been isolated from the different parts of bitter melon, including triterpenoids, proteins, lipids, steroids, monoterpenes, alkaloids, etc. Some of them were shown to have hypoglycaemic effects, especially triterpenoids and proteins (Chang et al., 2008, 2011; Cheng et al., 2008; Lo et al., 2014; Zhang et al., 2014), whereas the action modes and mechanisms of most of these hypoglycaemic molecules, and whether they work under insulin-resistant conditions, remain undefined.

In our previous study, constituents in the crude extract of the *M. charantia* stem were screened, and three structurally similar triterpenes, namely (23*E*)-cucurbita-5,23,25-triene-3β,7β-diol (CTD), 3β,25-dihydroxy-7β-methoxycucurbita-5,23(*E*)-diene (DHM; Fig. 1A), and 3β,7β,25-trihydroxycucurbita-5,23(*E*)-

Fig. 1 – Activation of the insulin-signalling pathway by DHM and THC in the presence or absence of insulin. (A) Structures of DHM and THC. (B and D) Western blot analysis of tyrosine-phosphorylated and total IRS-1 (P-Y-IRS and IRS, respectively), phosphorylated and total Akt (P-Akt and Akt, respectively), and actin in FL83B cells. The cells were pretreated using the vehicle or TNF-α, followed by stimulation using insulin, troglitazone (TZD), DHM, or THC for 30 min, as indicated underneath the blots. The assays for DHM and THC were performed in duplicate. (C) Western blot analysis of phosphorylated AS160 (P-AS160) and actin in FL83B cells treated as indicated underneath the blots. The band intensity of P-AS160 relative to Lane 1 was determined after normalization by that of actin. The data of the histogram represent the mean of two independent assays. The blots shown are from one of the experiments. (E) Western blot analysis of phosphorylated and total AS160, and actin in FL83B cells treated as indicated underneath the blots. Compound C was added with the chemical in Lanes 5–8. The band intensity of P-AS160 relative to Lane 1 was determined after normalization by that of AS160. (F) The glucose uptake assays. Cells were pretreated using TNF-α, followed by treatment using the vehicle (Groups 1 and 5) or the chemical as indicated. Compound C was added with the chemical in Groups 5–8. The relative glucose uptake of each group versus Group 1 was determined. The experiments were performed twice independently, each in triplicate. The data represent the mean ± standard deviations of the experiments. *$p < 0.05$ versus Group 1; #$p < 0.05$ between Groups 6 and 2, Groups 7 and 3, and Groups 8 and 4.