Original Article

Raspberry Ketone Promotes FNDC5 Protein Expression via HO-1 Upregulation in 3T3-L1 Adipocytes

Yung-Chieh Tsai[1,2,3]*, Jung-Hua Chen[4], Yen-Mei Lee[5], Mao-Hsiung Yen[5], Pao-Yun Cheng[4]*

[1]Department of Obstetrics and Gynecology, Chi-Mei Medical Center, Tainan, Taiwan, [2]Department of Medicine, Taipei Medical University, Taipei, Taiwan, [3]Department of Sport Management, Chia Nan University of Pharmacy and Science, Tainan, Taiwan, [4]Department of Physiology and Biophysics, National Defense Medical Center, Taipei, [5]Department of Pharmacology, National Defense Medical Center, Taipei, Taiwan

Abstract

Obesity is a global health problem and a risk factor for cardiovascular diseases and cancers. Exercise is an effective intervention to combat obesity. Fibronectin type III domain containing protein 5 (FNDC5)/irisin, a myokine, can stimulate the browning of white adipose tissue by increasing uncoupling protein 1 (UCP1) expression, and therefore may represent a link between the beneficial effects of exercise and improvement in metabolic diseases. Thus, upregulating the endogenous expression of FNDC5/irisin by administering medication would be a good approach for treating obesity. Herein, we evaluated the efficacy of raspberry ketone (RK) in inducing FNDC5/irisin expression and the underlying mechanisms. The expression of brown fat-specific proteins (PR domain containing 16 (PRDM16), CD137, and UCP1), heme oxygenase-1 (HO-1), FNDC5, and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) in differentiated 3T3-L1 adipocyte was analyzed by western blotting or immunofluorescence. The level of irisin in the culture medium was also assayed using an enzyme-linked immunosorbent assay kit. Results showed that RK (50 μM) significantly induced the upregulation of FNDC5 protein in differentiated 3T3-L1 adipocytes; however, the irisin level in the culture media was unaffected. Moreover, RK significantly increased the levels of PGC1α, brown adipocyte markers (PRDM16, CD137, and UCP1), and HO-1. Furthermore, the upregulation of PGC1α and FNDC5 and the browning effect induced by RK were significantly reduced by SnPP or FNDC5 siRNA, respectively. In conclusion, RK can induce FNDC5 protein expression via the HO-1 signaling pathway, and this study provides new evidence for the potential use of RK in the treatment of obesity.

Keywords: 3T3-L1 adipocyte, fibronectin type III domain containing protein 5, heme oxygenase-1, obesity, raspberry ketone

Introduction

Obesity is a manifestation of an energy imbalance in the body and has recently become a main public health problem.[1] It is considered to increase the incidence of chronic diseases, including cardiovascular diseases, Type 2 diabetes, and cancers.[2] Although changing diet and adopting a lifestyle with increased physical activity are the first line of treatment for obesity, it is difficult for ill or disabled people to maintain these lifestyle changes. Thus, finding a novel strategy for the prevention and treatment of obesity is necessary.

Although research of therapeutic drugs with efficacies against obesity is increasing, regular physical exercise is almost always the first recommendation for the prevention and treatment of obesity.[3] Many pieces of evidence have demonstrated that the benefits of exercise are mediated by irisin, which is produced from the cleavage of the extracellular portion of fibronectin type III domain containing protein 5 (FNDC5). FNDC5 has been observed in the skeletal muscles, liver, brain, and adipose tissues.[4,5] In addition, several studies have indicated that FNDC5/irisin participates in metabolic processes. In an *in vivo* study, recombinant irisin administration or adenoviral overexpression of FNDC5 significantly ameliorated glucose

Received: 03-Nov-2021 **Revised:** 13-Jan-2022 **Accepted:** 25-Feb-2022
Published: 28-Apr-2022

Access this article online

Quick Response Code:

Website: www.cjphysiology.org

DOI: 10.4103/cjp.cjp_95_21

***Address for correspondence:** Dr. Yung-Chieh Tsai, Department of Obstetrics and Gynecology, Chi-Mei Medical Center, No.901, Zhonghua Rd. Yongkang Dist., Tainan 71004, Taiwan.
E-mail: yung0613@ms2.hinet.net
Prof. Pao-Yun Cheng, Department of Physiology and Biophysics, Graduate Institute of Physiology, National Defense Medical Center, No. 161, Sec 6, Minquan East Road, Taipei, Taiwan.
E-mail: pycheng@mail.ndmctsgh.edu.tw

How to cite this article: Tsai YC, Chen JH, Lee YM, Yen MH, Cheng PY. Raspberry ketone promotes FNDC5 protein expression via HO-1 upregulation in 3T3-L1 adipocytes. Chin J Physiol 2022;65:80-6.

metabolism in obese mice by increasing the level of irisin in circulation.[6,7] In an *in vitro* study, FNDC5/irisin enhanced adipocyte browning, with the cell morphology showing a higher density of mitochondria and multilocular lipid droplets.[6] Therefore, FNDC5/irisin may be a key protein in the treatment of metabolic diseases with exercise. Moreover, treatment with phytochemicals or medications to stimulate endogenous FNDC5/irisin production may be a good strategy, in particular for people who could not exercise regularly.

Raspberry ketone (4-(4-hydroxyphenyl)-2-butanone, RK) is a phytochemical present in European red raspberry *Rubus idaeus*.[8] Owing to its multiple health benefits, RK has received increasing attention. RK has been established to reduce adipogenesis in adipocytes,[9,10] exerting an efficacy against obesity in rodents.[8,9,11] Furthermore, in a recent study, raspberry supplementation induced the browning of white adipose tissue in mice and elevated peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) levels and FNDC5/irisin content.[12] In this study, we investigated whether RK is an FNDC5/irisin inducer in differentiated 3T3-L1 adipocytes and clarified the underlying molecular targets. The results from this study can be used as reference for subsequent RK research applications, such as the prevention and treatment of obesity.

Materials and Methods

3T3-L1 cell culture, differentiation, and treatment

3T3-L1 mouse embryo fibroblasts (#BCRC60159) were purchased from the Food Industry Research and Development Institute (Hsinchu, Taiwan). The cells were differentiated as described previously.[8] Briefly, once confluent, the cells were further cultured in maintenance medium (Dulbecco modified Eagle medium (DMEM) supplemented with 10% bovine calf serum) for 2 days (day 0), and then cultured in differentiation medium (DMEM supplemented with 10% fetal bovine serum plus MDI that contained 0.5 mM 3-isobutyl-1-Methylxanthine, 1 μM Dexamethasone, and 10 μg/ml Insulin) for 3 days. After that, the cells were cultured in maintenance medium containing insulin (10 μg/ml) for 6 days. Next, differentiated, mature cells were treated with RK (10–100 μM; Cat. number: 178519, purity ≥98.5%, gas chromatography, GC; Sigma-Aldrich, St. Louis, MO, USA) for 24 h in the presence or absence of the heme oxygenase-1 (HO-1) inhibitor tin protoporphyrin-IX (SnPP; 20 μM; Tocris Bioscience, Bristol, UK).[8] The harvested cells were used in subsequent experiments.

Measurement of cell viability

Cell survival was evaluated using a 3-[4,5-dimethylthiazol-2-yl]-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (a MTS tetrazolium compound)-based CellTiter 96® AQueous One Solution (Promega, Madison, WI, USA). Briefly, cells were treated with RK at 10–300 μM for 24 h in a 96-well plate. MTS reduction was started by adding MTS solution 20 μl per well. Plates were incubated at 37°C. After 4 h incubation, the reaction was stopped by adding 25 μl of 10% SDS. Absorbance was determined at 490 nm using a microplate reader (Model 550, BIO-RAD Laboratories, CA, USA) in accordance with the manufacturer's instructions.

Irisin level in the culture medium

After incubation with RK for 24 h, the cell culture medium in each well was collected for measuring the level of released irisin. Irisin level was evaluated using a commercial irisin Enzyme-Linked Immunosorbent Assay (ELISA) kit (AG-45A-0046YEK-K10; AdipoGen Life Sciences, Switzerland) according to the manufacturer's instructions. Microplate analysis was conducted using an ELISA reader at a wavelength of 450 nm to obtain concentration values.

Knockdown of fibronectin type III domain containing protein 5 by small interference RNA transfection

FNDC5 small interference RNA (siRNA) (sc-145214) and a non-related control siRNA (sc-36869) were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). On day 9, mature adipocytes were transfected with validated siRNA at a final concentration of 20 nM in the presence of the transfection reagent Lipofectamine™ 3000 (Thermo Fisher Scientific, Waltham, MA, USA) for 6 h, according to the manufacturer's protocol. Thereafter, the transfected cells were incubated with RK (50 μM) for 24 h and then harvested.

Western blotting

Cells were lysed in ice-cold lysis buffer, and the lysates were centrifuged at 12,000 × g at 4°C for 20 min. The sample concentration was evaluated by a Pierce BCA Protein Assay kit (Thermo Fisher Scientific, MA, USA). Proteins were separated on SDS-polyacrylamide gels and then blotted onto membranes.[8] The blots were then reacted overnight at 4°C with the following primary antibodies: mouse anti-β-actin monoclonal antibody (A1978; Sigma-Aldrich), rabbit anti-CD137 monoclonal antibody (sc-398933; Santa Cruz Biotechnology, Inc.), mouse anti-HO-1 monoclonal antibody (ADI-OSA-110; Enzo Life Sciences, NY, USA), rabbit anti-FNDC5 monoclonal antibody (ab174833; Abcam, Cambridge, UK), goat anti-PGC1α polyclonal antibody (ab106814; Abcam), rabbit anti-PR domain containing 16 (PRDM16) polyclonal antibody (ab106410; Abcam), rabbit anti-uncoupling protein 1 (UCP1) polyclonal antibody (ab10983; Abcam), and mouse anti-α-tubulin monoclonal antibody (#3873; Cell Signaling Technology, Danvers, MA, USA). Subsequently, the blot membranes were reacted with horseradish peroxidase-labeled secondary antibody for 1 h and further analyzed using enhanced chemiluminescence reagents (Pierce).[8]

Immunofluorescence

Cells were stained for immunofluorescence as described previously.[8] Briefly, cells were grown on μ-dish (Martinsried, IBIDI, Germany) and stained with 500 nM Mitotracker deep red FM in serum-free DMEM for 30 min at 37°C according to the manufacturer's instructions. Next, the cells were washed with phosphate buffered saline (PBS) and fixed with 4% paraformaldehyde. Thereafter, the cells were blocked with 5% bovine serum albumin in PBS at 25°C for 1 h and then

reacted with primary anti-UCP1 polyclonal antibody (ab10983; 1:1000; Abcam) overnight at 4°C. Subsequently, the cells were reacted with secondary antibody Alexa Fluor 488 (ab150077; 1:1000; Abcam) at room temperature for 1 h. Nuclei were counterstained with DAPI (#4083; Cell Signaling Technology) for 5 min. Finally, images were obtained under a fluorescence light microscope (Nikon Eclipse Ti-E; Nikon, Kawasaki, Japan).

Statistical analysis

The data are presented as mean ± standard error of the mean. Statistical analysis was performed using SigmaPlot 12.5 (Systat Software, Inc., San Jose, CA, USA). Differences between means were determined using one-way analysis of variance followed by Newman–Keuls multiple comparison *post hoc* test. Differences were considered statistically significant at a *P-value* of < 0.05.

Results

Effects of raspberry ketone on the expression of fibronectin type III domain containing protein 5/irisin in 3T3-L1 adipocytes

First, the effect of various concentrations (10–300 μM) of RK on the cytotoxicity of 3T3-L1 adipocytes was examined using the MTS test. There was no significant cytotoxicity when 3T3-L1 adipocytes were treated with RK at concentrations below 200 μM [Figure 1a]. Therefore, the range of concentrations of 10–100 μM RK was selected for further studies, and the results showed that RK (25 and 50 μM) significantly increased the level of FNDC5 protein in differentiated 3T3-L1 adipocytes [$P < 0.05$; Figure 1b]. Notably, the level of FNDC5 protein expression was significantly increased by 24% by 50 μM RK, but the levels of irisin in the culture medium did not significantly change [Figure 1c].

Involvement of heme oxygenase-1 in the upregulation of fibronectin type III domain containing protein 5 in differentiated 3T3-L1 adipocytes

We investigated the molecular targets that mediate the modulation of the expression of FNDC5, HO-1, and PGC1α in RK-treated differentiated 3T3-L1 adipocytes. The data showed that RK significantly increased the levels of HO-1 and PGC1α [$P < 0.05$; Figure 2a and b]. To further clarify the role of HO-1 in the upregulation of PGC1α/FNDC5 induced by RK, its activity was inhibited with SnPP. Results showed that the elevation of HO-1 level was further enhanced after treatment with RK (50 μM) and SnPP (20 μM) compared with that in the group treated with RK alone [Figure 2c]. In contrast, RK (50 μM)-induced upregulation of PGC1α and FNDC5 was significantly reduced in differentiated 3T3-L1 cells pretreated with SnPP (20 μM) [$P < 0.05$; Figure 2d and e].

Effects of raspberry ketone on the expression of brown adipocyte markers and the effect validation by fibronectin type III domain containing protein 5 knockdown

To clarify whether FNDC5 mediated the effect of RK, the levels of brown adipocyte markers in differentiated 3T3-L1 adipocytes were analyzed by western blotting. RK significantly elevated the level of brown adipocyte markers (PRDM16, CD137, and UCP1; Figure 3a-c) in differentiated 3T3-L1 adipocytes. Notably, the level of the brown adipocyte markers increased by approximately 30%–40% after treatment with RK, compared with that in the control group ($P < 0.05$). Furthermore, the RK-induced upregulation of cellular UCP1 expression was confirmed by immunofluorescent staining [Figure 3d]. On the contrary, the effect of RK on the expression of FNDC5 and brown adipocyte markers was significantly reduced after treatment with FNDC5 siRNA, compared with that in the siNeg-treated group [$P < 0.05$; Figure 4].

Discussion

To the best of our knowledge, this is the first study to reveal that RK induces FNDC5 expression in differentiated 3T3-L1 adipocytes. Our results showed that RK significantly increased the level of FNDC5 protein and might be associated with the upregulation of HO-1/PGC1α. In addition, the HO-1/PGC1α/FNDC5 signaling pathway mediated the browning effect of RK in differentiated 3T3-L1 adipocytes [Figure 5]. These findings

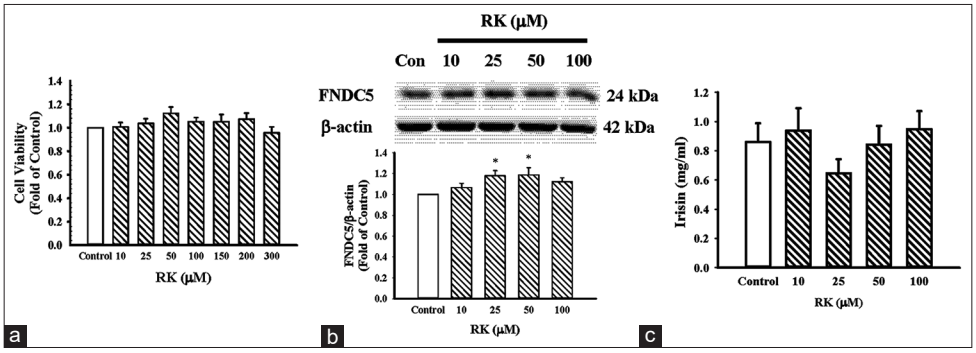

Figure 1: Raspberry ketone increased the expression of fibronectin type III domain containing protein 5 protein. (a) Effects of raspberry ketone on cells viability. 3T3-L1 cells were treated with raspberry ketone for 24 h, and cell viability was measured by the MTS assay. The values are mean ± standard error of the mean for three independent experiments. *$P < 0.05$ compared with the control. (b) Effect of raspberry ketone on the level of fibronectin type III domain containing protein 5 protein in differentiated 3T3-L1 adipocytes was analyzed by western blotting. (c) Effect of raspberry ketone on the levels of irisin in the culture medium was evaluated by enzyme-linked immunosorbent assay. Data are expressed as mean ± standard error of the mean ($n = 3$). *$P < 0.05$ compared with the control.

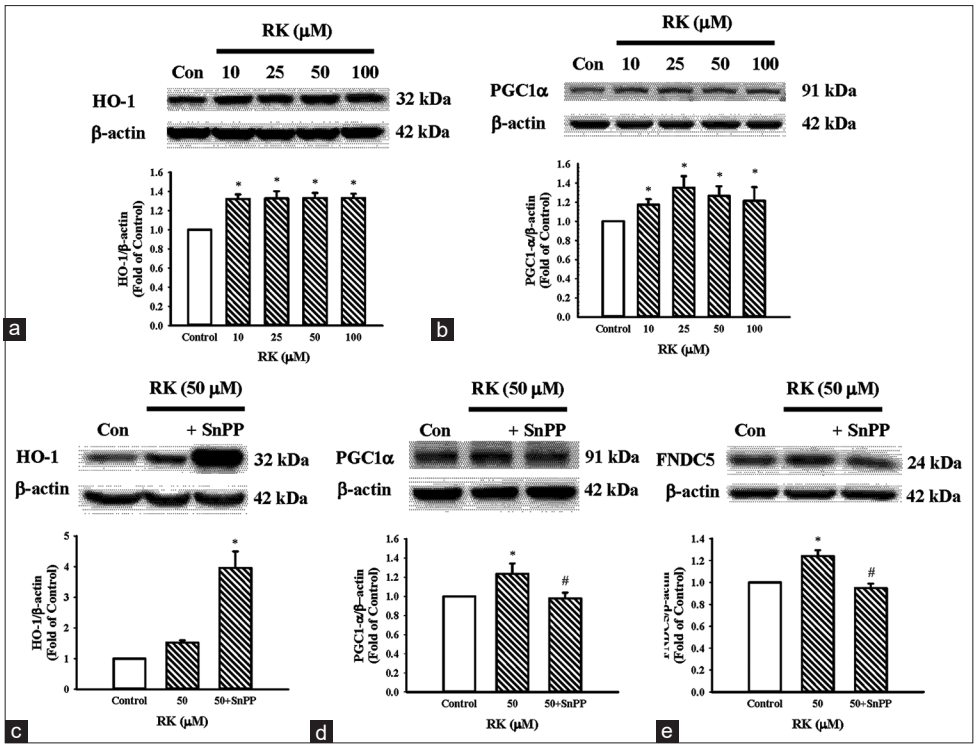

Figure 2: Heme oxygenase-1 is involved in raspberry ketone-induced fibronectin type III domain containing protein 5 expression in differentiated 3T3-L1 adipocytes. (a and b) Effect of raspberry ketone on the level of heme oxygenase-1 and peroxisome proliferator-activated receptor gamma coactivator 1-alpha proteins in differentiated 3T3-L1 adipocytes. (c-e) Modulation of heme oxygenase-1, peroxisome proliferator-activated receptor gamma coactivator 1-alpha, and fibronectin type III domain containing protein 5 expression by SnPP (20 μM) in raspberry ketone-treated differentiated 3T3-L1 adipocytes. Data are expressed as mean ± standard error of the mean ($n = 3$). *$P < 0.05$ compared with the control; #$P < 0.05$ compared with the group treated with raspberry ketone alone.

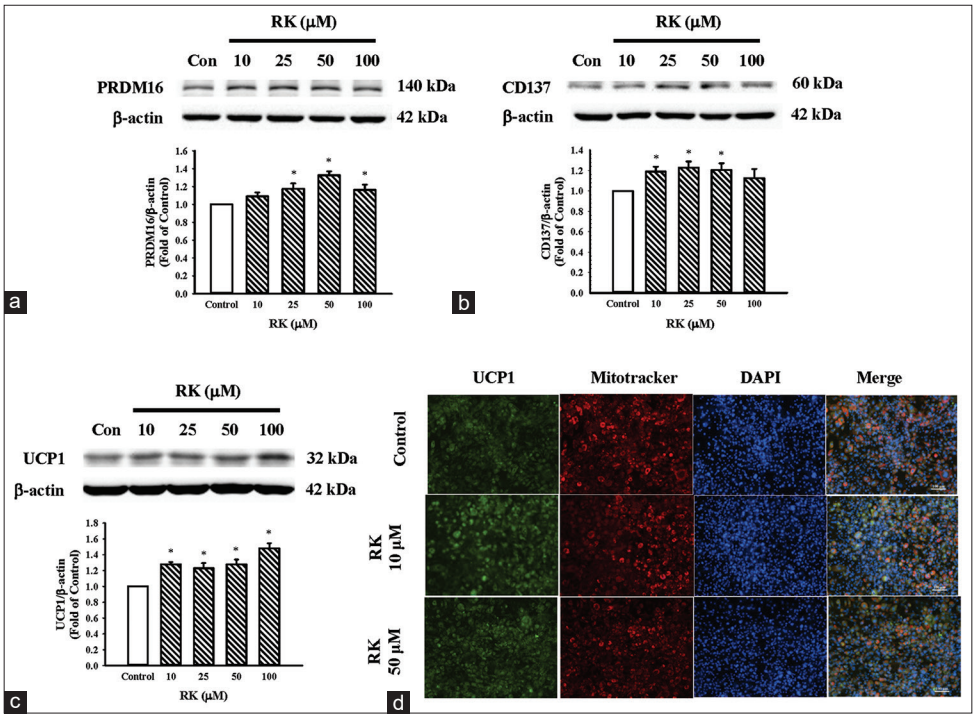

Figure 3: Raspberry ketone induced the browning of differentiated 3T3-L1 adipocytes. Western blotting analysis of the brown adipocyte markers (a) PR domain containing 16, (b) CD137, and (c) uncoupling protein 1 in raspberry ketone-treated differentiated 3T3-L1 adipocytes. Data are expressed as mean ± standard error of the mean ($n = 3$–4). *$P < 0.05$ compared with the control. (d) Immunofluorescence staining for uncoupling protein 1 and MitoTracker Deep Red FM in raspberry ketone-treated differentiated 3T3-L1 cells. The scale bars are 100 μm.

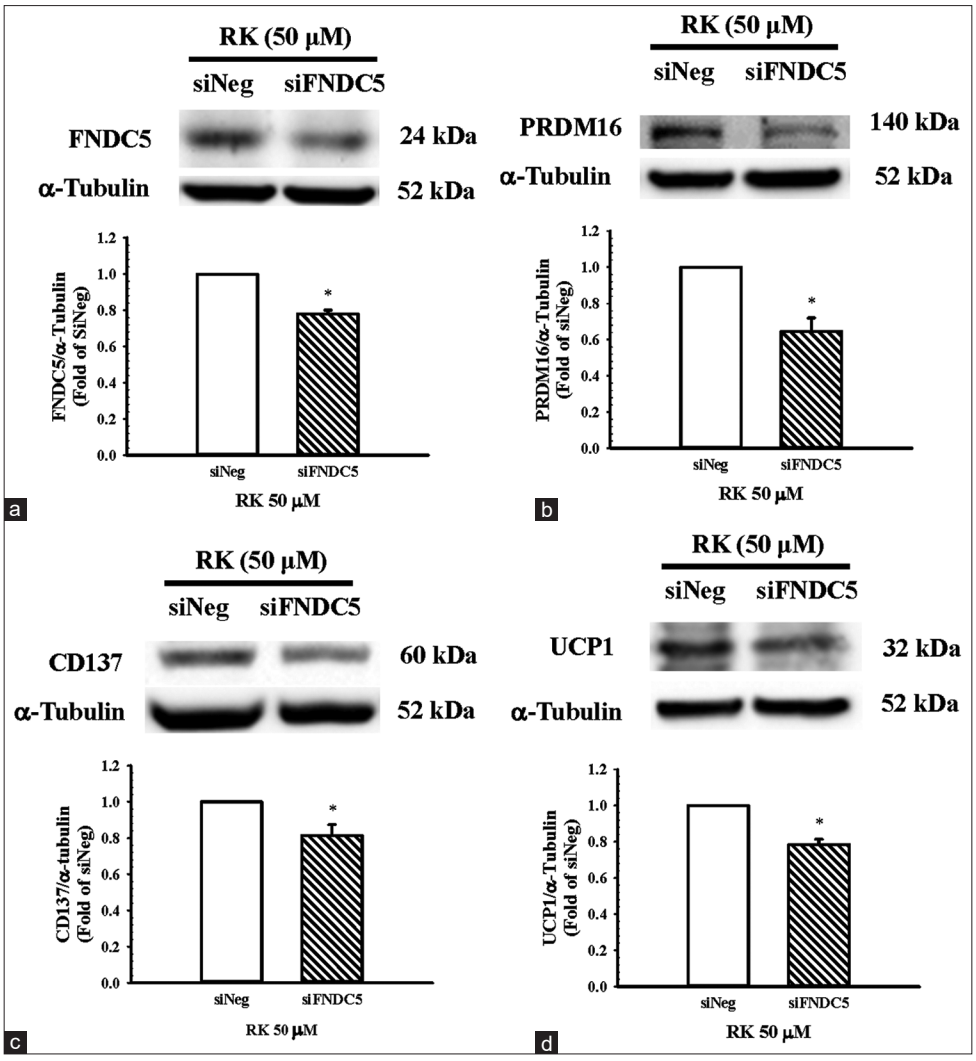

Figure 4: Fibronectin type III domain containing protein 5 is involved in raspberry ketone-induced browning effect of differentiated 3T3-L1 adipocytes. Western blotting analysis of (a) fibronectin type III domain containing protein 5 and the brown adipocyte markers, (b) PR domain containing 16, (c) CD137, and (d) uncoupling protein 1 in raspberry ketone-treated differentiated 3T3-L1 adipocytes under fibronectin type III domain containing protein 5 knockdown by FNDC5 siRNA. Data are expressed as mean ± standard error of the mean ($n = 3$). *$P < 0.05$ compared with the negative siRNA group. siNeg, negative siRNA; siFNDC5, Fibronectin type III domain containing protein 5 siRNA.

provide a new evidence supporting the potential use of RK in the therapy of obesity.

Accumulating evidence has shown that exercise-induced upregulation of FNDC5 expression and the subsequent production of irisin is brought about by the proteolytic cleavage of FNDC5.[5] Irisin can trigger white fat cell transfer into brown fat cells via an unknown receptor of adipocyte.[11] Moreover, FNDC5 overexpression leads to an increase in the circulating levels of irisin and stimulates the browning of white inguinal adipose tissue in mice.[5] Considering these beneficial effects in ameliorating the metabolic profile, irisin is a promising target for the prevention and treatment of obesity.[12] In contrast to these findings, a study reported that FNDC5 content in the muscle and irisin levels in circulation were not significantly affected by chronic endurance training.[13] Alternatively, chronic endurance exercise-induced browning of subcutaneous inguinal fat was mediated by locally produced FNDC5, not by circulating irisin.[13] Similar to the above results, our data showed that RK significantly induced the upregulation of FNDC5 in differentiated 3T3-L1 adipocytes. Moreover, the browning effect of RK was associated with FNDC5 protein expression, but did not correlate to irisin level in the culture medium [Figure 4]. This phenomenon may be explained by the hypothesis that tight dimerization of the FNDC5 ectodomain may cause the formation of dimer at the cell surface, which can lead to autocrine or paracrine signaling, and this effect is independent of irisin.[14] These results suggest that RK is a FNDC5 inducer and may be a good candidate for the therapy of obesity.

Many studies have shown that FNDC5 expression is regulated by PGC1α in skeletal muscle and neurons.[5,15] PGC1α is also a transcriptional factor, and it plays an essential role in

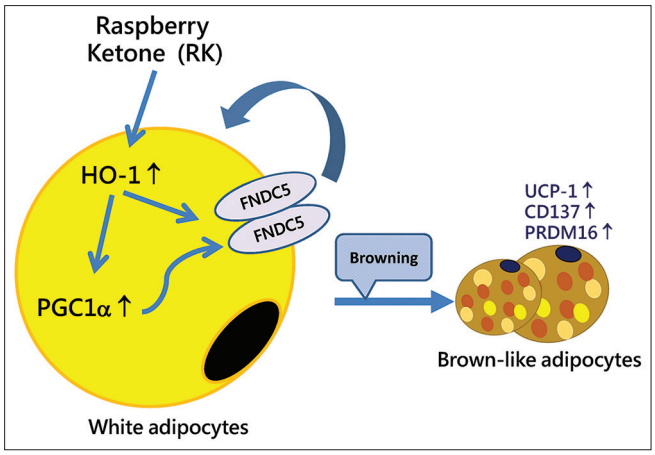

Figure 5: Suggested pathways associated with raspberry ketone-induced adipocyte browning via the heme oxygenase-1/fibronectin type III domain containing protein 5 pathway. The action of fibronectin type III domain containing protein 5 protein is a required second signal promoting browning proteins expression in raspberry ketone-treated adipocyte. Raspberry ketone increased the level of fibronectin type III domain containing protein 5 protein and might be associated with the upregulation of heme oxygenase-1 and/or peroxisome proliferator-activated receptor gamma coactivator 1-alpha. (→) stimulatory, (↑) upregulation.

thermogenesis,[16] mitochondrial functions, and cellular energy metabolism.[17] In addition, an increasing number of studies have reported that HO-1, a stress-responsive enzyme, can protect rodents from obesity and modulate adipocyte stem cell differentiation.[18-20] Moreover, the role of HO-1 in maintaining beige-like adipose tissue and improving cardiovascular and liver functions has been reported.[21,22] Notably, HO-1 expression is mediated via PGC1α activation, and it can further indirectly increase PGC1α levels.[23] These findings suggest that the induction of PGC1α/FNDC5 is also dependent on HO-1 expression. Consistent with these studies, our study revealed that RK increased HO-1 and PGC1α expression in differentiated 3T3-L1 adipocytes [Figure 2]. Furthermore, SnPP, a specific HO-1 inhibitor, blocked the upregulation of PGC1α and FNDC5 induced by RK. These results indicated that the positive regulation of FNDC5 by HO-1 may also be a common mechanism involved in FNDC5 induction after RK treatment, and further studies are needed to clarify the role of PGC1α in the regulation of FNDC5 by RK. It is noteworthy that HO-1 expression further increased after treatment with SnPP, and this can be due to the enzyme feedback inhibition, confirming the findings of previous studies.[24-26] However, further studies are required to clarify the exact mechanism of FNDC5/irisin activity in adipocytes by a receptor-like mediating pathway.

It is noteworthy that the activation of HO-1 and brown adipocyte markers by RK (25–100 μM), but RK alone had a biphasic effect on FNDC5. A relatively low concentration of 25 μM RK significantly increased FNDC5 expression, whereas 100 μM RK produces less effect. Although we have no direct evidence of the differences of RK on this phenomenon, it is plausible that (1) RK can increase the FNDC5 proteins expression indirectly, possibly by activating or inhibiting another factor, to decrease these patterns; (2) the expression of brown adipocyte markers induced by RK may via other mechanism.[27] Further studies are needed to clarify the mechanism of different concentration of RK.

In this study, we found the effect of RK is mediated by FNDC5, not by its cleaved peptide irisin. Similar phenomenon were also found in other studies, the beneficial effects of FNDC5 induced by either exogenous FNDC5 or FNDC5 overexpression, it is still difficult to unmask the probable effectors of endogenous FNDC5 and its cleaved fragment, irisn.[28-30] Many questions remain regarding generation and quantitation of endogenous irisin, because the data on irisin levels based on ELISAs are compromised by major methodical problems,[31] which is a limitation in the present study. Although we had shown RK had browning effect *in vivo* and *in vitro* studies,[8,27,32] this is the first study revealed that RK is an inducer of FNDC5 protein in differentiated 3T3-L1 adipocytes. In this study, we did not test whether RK administration increases circulating irisin levels and FNDC5 expression in an animal model, therefore, additional experiments are needed to elucidate the inter-relationship between RK, HO-1, and FNDC5 in an *in vivo* setting. Furthermore, the role of HO-1 in RK-induced FNDC5 expression should be validated in specific HO-1-knockdown mice in future studies.

Conclusion

In conclusion, RK can modulate the expression of FNDC5 protein via HO-1 and subsequently induce the browning of differentiated 3T3-L1 adipocytes. This study provides a mechanistic understanding of the beneficial effects of RK and reinforces the value of RK as a potential agent for the therapy of obesity.

Financial support and sponsorship

This study was partially supported by a research grant from the Ministry of Science and Technology (MOST-108-2320-B-016-016-MY3), Ministry of National Defense Affairs Bureau (MAB-109-009), and Chi-Mei Medical Center (CMNDMC10903), Taiwan.

Conflicts of interest

There are no conflicts of interest.

References

1. Voss JD, Pavela G, Stanford FC. Obesity as a threat to national security: The need for precision engagement. Int J Obes (Lond) 2019;43:437-9.
2. Romieu I, Dossus L, Barquera S, Blottière HM, Franks PW, Gunter M, *et al.* Energy balance and obesity: What are the main drivers? Cancer Causes Control 2017;28:247-58.
3. Gamas L, Matafome P, Seiça R. Irisin and myonectin regulation in the insulin resistant muscle: Implications to adipose tissue: Muscle crosstalk. J Diabetes Res 2015;2015:359159.
4. Zhang J, Zhang W. Can irisin be a linker between physical activity and brain function? Biomol Concepts 2016;7:253-8.
5. Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, *et al.* A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 2012;481:463-8.

6. Zhang Y, Li R, Meng Y, Li S, Donelan W, Zhao Y, *et al.* Irisin stimulates browning of white adipocytes through mitogen-activated protein kinase p38 MAP kinase and ERK MAP kinase signaling. Diabetes 2014;63:514-25.
7. Morimoto C, Satoh Y, Hara M, Inoue S, Tsujita T, Okuda H. Anti-obese action of raspberry ketone. Life Sci 2005;77:194-204.
8. Tsai YC, Yang BC, Peng WH, Lee YM, Yen MH, Cheng PY. Heme oxygenase-1 mediates anti-adipogenesis effect of raspberry ketone in 3T3-L1 cells. Phytomedicine 2017;31:11-7.
9. Kshatriya D, Li X, Giunta GM, Yuan B, Zhao D, Simon JE, *et al.* Phenolic-enriched raspberry fruit extract (*Rubus idaeus*) resulted in lower weight gain, increased ambulatory activity, and elevated hepatic lipoprotein lipase and heme oxygenase-1 expression in male mice fed a high-fat diet. Nutr Res 2019;68:19-33.
10. Xing T, Kang Y, Xu X, Wang B, Du M, Zhu MJ. Raspberry supplementation improves insulin signaling and promotes brown-like adipocyte development in white adipose tissue of obese mice. Mol Nutr Food Res 2018;62:1701035.
11. Sanchez-Delgado G, Martinez-Tellez B, Olza J, Aguilera CM, Gil Á, Ruiz JR. Role of exercise in the activation of brown adipose tissue. Ann Nutr Metab 2015;67:21-32.
12. Castillo-Quan JI. From white to brown fat through the PGC-1α-dependent myokine irisin: Implications for diabetes and obesity. Dis Model Mech 2012;5:293-5.
13. Wu MV, Bikopoulos G, Hung S, Ceddia RB. Thermogenic capacity is antagonistically regulated in classical brown and white subcutaneous fat depots by high fat diet and endurance training in rats: Impact on whole-body energy expenditure. J Biol Chem 2014;289:34129-40.
14. Schumacher MA, Chinnam N, Ohashi T, Shah RS, Erickson HP. The structure of irisin reveals a novel intersubunit β-sheet fibronectin type III (FNIII) dimer: Implications for receptor activation. J Biol Chem 2013;288:33738-44.
15. Wrann CD, White JP, Salogiannnis J, Laznik-Bogoslavski D, Wu J, Ma D, *et al*. Exercise induces hippocampal BDNF through a PGC-1α/FNDC5 pathway. Cell Metab 2013;18:649-59.
16. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell 1998;92:829-39.
17. Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, *et al.* Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 1999;98:115-24.
18. Cao J, Inoue K, Sodhi K, Puri N, Peterson SJ, Rezzani R, *et al.* High-fat diet exacerbates renal dysfunction in SHR: Reversal by induction of HO-1-adiponectin axis. Obesity (Silver Spring) 2012;20:945-53.
19. Li M, Kim DH, Tsenovoy PL, Peterson SJ, Rezzani R, Rodella LF, *et al.* Treatment of obese diabetic mice with a heme oxygenase inducer reduces visceral and subcutaneous adiposity, increases adiponectin levels, and improves insulin sensitivity and glucose tolerance. Diabetes 2008;57:1526-35.
20. Vanella L, Sodhi K, Kim DH, Puri N, Maheshwari M, Hinds TD, *et al.* Increased heme-oxygenase 1 expression in mesenchymal stem cell-derived adipocytes decreases differentiation and lipid accumulation via upregulation of the canonical Wnt signaling cascade. Stem Cell Res Ther 2013;4:28.
21. Abraham NG, Kappas A. Pharmacological and clinical aspects of heme oxygenase. Pharmacol Rev 2008;60:79-127.
22. Abraham NG, Junge JM, Drummond GS. Translational significance of heme oxygenase in obesity and metabolic syndrome. Trends Pharmacol Sci 2016;37:17-36.
23. Singh SP, Schragenheim J, Cao J, Falck JR, Abraham NG, Bellner L. PGC-1 alpha regulates HO-1 expression, mitochondrial dynamics and biogenesis: Role of epoxyeicosatrienoic acid. Prostaglandins Other Lipid Mediat 2016;125:8-18.
24. Sardana MK, Kappas A. Dual control mechanism for heme oxygenase: Tin(IV)-protoporphyrin potently inhibits enzyme activity while markedly increasing content of enzyme protein in liver. Proc Natl Acad Sci U S A 1987;84:2464-8.
25. Zager RA. Marked protection against acute renal and hepatic injury after nitrited myoglobin + tin protoporphyrin administration. Transl Res 2015;166:485-501.
26. Zager RA, Johnson AC, Frostad KB. Combined iron sucrose and protoporphyrin treatment protects against ischemic and toxin-mediated acute renal failure. Kidney Int 2016;90:67-76.
27. Leu SY, Tsai YC, Chen WC, Hsu CH, Lee YM, Cheng PY. Raspberry ketone induces brown-like adipocyte formation through suppression of autophagy in adipocytes and adipose tissue. J Nutr Biochem 2018;56:116-25.
28. Geng Z, Fan WY, Zhou B, Ye C, Tong Y, Zhou YB, *et al.* FNDC5 attenuates obesity-induced cardiac hypertrophy by inactivating JAK2/STAT3-associated inflammation and oxidative stress. J Transl Med 2019;17:107.
29. Liu H, Wang M, Jin Z, Sun D, Zhu T, Liu X, *et al.* FNDC5 induces M2 macrophage polarization and promotes hepatocellular carcinoma cell growth by affecting the PPARγ/NF-κB/NLRP3 pathway. Biochem Biophys Res Commun 2021;582:77-85.
30. Zhou B, Qiu Y, Wu N, Chen AD, Zhou H, Chen Q, *et al.* FNDC5 Attenuates oxidative stress and NLRP3 inflammasome activation in vascular smooth muscle cells via activating the AMPK-SIRT1 signal pathway. Oxid Med Cell Longev 2020;2020:6384803.
31. Maak S, Norheim F, Drevon CA, Erickson HP. Progress and challenges in the biology of FNDC5 and irisin. Endocr Rev 2021;42:436-56.
32. Leu SY, Chen YC, Tsai YC, Hung YW, Hsu CH, Lee YM, *et al.* Raspberry ketone reduced lipid accumulation in 3T3-L1 Cells and ovariectomy-induced obesity in wistar rats by regulating autophagy mechanisms. J Agric Food Chem 2017;65:10907-14.