Long chain fatty acid induces autophagy via AMPK/ULK1 signaling pathway resulting in energy metabolism imbalance in cardiomyoblast cells.

Yi-Chieh Tsai [1], Wei- Wen Kuo [4], Chih-Yang Huang [1, 2, 3]

[1.] *Graduate Institute of Basic Medical Science, China Medical University, Taichung, Taiwan*
[2.] *Department of Health and Nutrition Biotechnology, Asia University, Taichung, Taiwan*
[3.] *Graduate Institute of Chinese Medical Science, China Medical University, Taichung, Taiwan*
[4.] *Department of Biological Science and Technology, China Medical University, Taichung*

Abstract

Background:

Metabolic syndromes increase the risk of developing cardiovascular disease. The risk factors for development of Metabolic Syndrome are elevated blood pressure, High fasting blood sugar, high serum triglycerides, low high-density lipoprotein (HDL) levels, and obesity. Acute toxicity from accumulation of long-chain fatty acids may lead to cell dysfunction and results in heart failure and diabetic cardiomyopathy, a phenomenon known as lipotoxicity. The occurrence of lipotoxicity induces energy imbalance. Cardiomyocytes use fatty acid as well as glucose for energy production. These substrates are transported into the cell by fatty acid transporter CD36 and the glucose transporter 4 (GLUT4). These two receptors have switching phenomenon, in this work we focus on how palmitic acid induces traffic and switch between these two receptors. On the other hand, autophagy is critical for cell survival during states of energy crisis. Recent study showed that palmitic acid induces autophagy, but the mechanism is still unclear. Otherwise, HDL was evidenced that therapies intended to reduce the risk of cardiovascular disease. Recent study demonstrated that HDL could activate PI3K-Akt-mTOR signaling which negatively regulates autophagy and related with energy flux. In this study, we investigated the HDL reverse energy metabolism imbalance by switching CD36 and GLUT4 signaling pathway and protects mTOR-independent signaling pathway in palmitic acid-induced autophagy .

Materials and Methods:

To understand how palmitic acid induces energy imbalance , switching of CD36 to GLUT4, autophagy and apoptosis, H9c2 cardiacmyoblast cells were treated with palmitic acid for 24hr. The western blotting, ELISA, MTT, Puncta assay, Lysotracker and Flow cytometry were then performed and the results were interpreted.

Results:

From the western blot and ELISA, we found palmitic acid induced the imbalance in energy metabolism and the switching of CD36 to GLUT4. HDL reverses palmatic acid-induced energy metabolism imbalance in H9c2 cardiomyoblast cells. Cardiomyocyte autophagy is activated under a wide range of pathological conditions. The western blot data showed that the autophagy marker were increased in Diabetes and Obesity rats. In addition, we found that mTOR-independent signaling pathway is involved in palmitic acid-induced autophagy to mediate cell death in H9c2 cardiacmyoblast cells. Conversely, HDL modulated mTOR-independent signaling pathway in palmitic acid-induced autophagy and result in cell survival.

Results

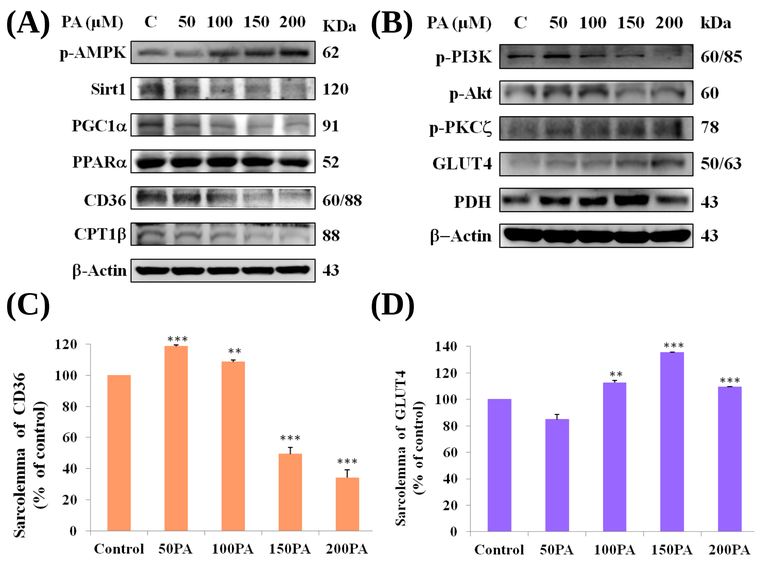

Fig. 1. Effects of palmitic acid on autophagy in H9c2 cardiomyoblast cells.

(A, B) Western blot was used to analysis total cell lysate from H9c2 cells that were treated with different dosages of palmitic acid (50μM、100 μM、150 μM、200 μM) for 24 hr. (C, D) ELISA assay of membrane proteins, H9c2 cells were treated with different dosages of palmitic acid for 24 hr. Data are presented as the mean ± SEM (n=3). ***P<0.001 and **P<0.01, represent significant difference to that of the control.

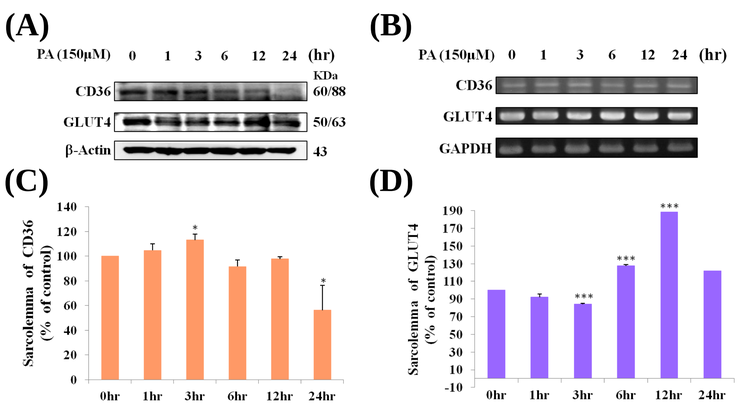

Fig. 2. Effects of palmitic acid on autophagy in H9c2 cardiomyoblast cells.

(A) H9c2 cells were treated with different dosages of palmitic acid (50μM、100 μM、150 μM、200 μM) for 24 hr., Western blot was used to analysis total cell lysates. (B, C) Puncta assay, after transfect with GFP-LC3 for 24 hr, H9c2 cells were treated with palmitic acid for 24 hr. Data are presented as the mean ± SEM (n=3). ***P<0.001 and **P<0.01, represent significantly difference versus with control. ##P<0.01, represent significantly difference versus with palmitic acid.

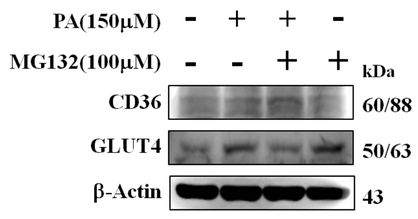

Fig. 3. Palmatic acid treatment influences the protein level of membrane proteins.

After treated with MG132 (100μM) for 9 hr, H9c2 cells were treated with 150μM palmitic acid for 24hr. Palmitic acid inhibited the CD36 and induced GLUT4 proteins level.

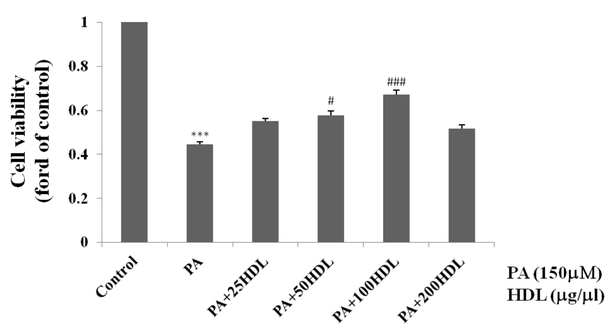

Fig. 4. The protective effect of HDL on palmatic acid-induced apoptosis in H9c2 cardiomyoblast cells.

After treatement with different dosages of HDL for 24 hr, H9c2 cells were treated with 150μM palmitic acid for 24hr. Data is presented as the mean ± S.E.M. (n=3). ***P<0.001, represent significantly difference versus the control. ###P<0.001 and #P<0.001, represent significantly difference with palmitic acid administration.

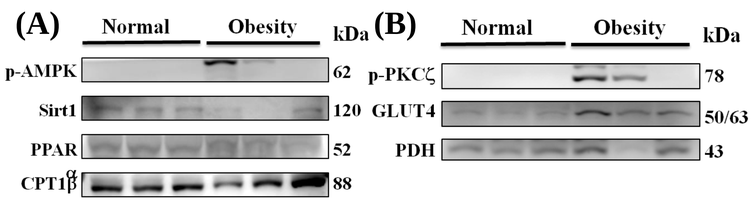

Fig. 5. Western blot analysis of energy metabolism pathway from the obesity rats myocardials.

The western blot was used to analyses (A) fatty acid pathway and (B) glucose pathway in obesity rats myocardial.

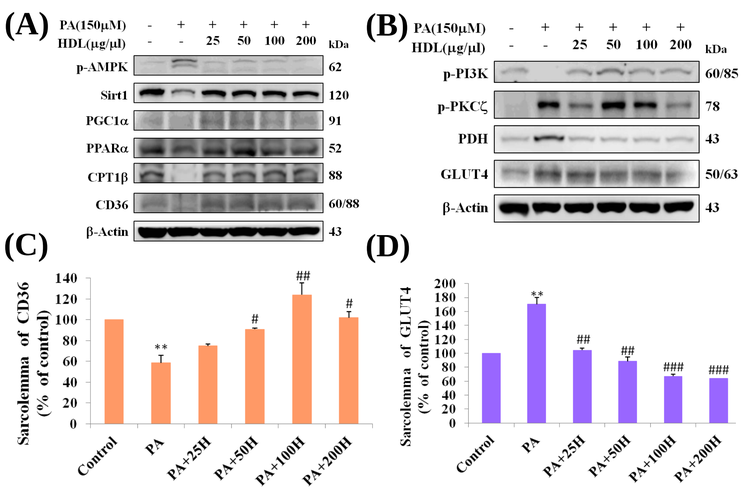

Fig. 6. The protective effect of HDL on palmatic acid-induced apoptosis in H9c2 cardiomyoblast cells.

After treatement with different dosages of HDL for 24 hr, H9c2 cells were treated with 150μM palmitic acid for 24hr. Data are presented as the mean ± S.E.M. (n=3). ***P<0.001, represent significantly difference versus control. ###P<0.001 and #P<0.001, represent significantly difference with palmitic acid.

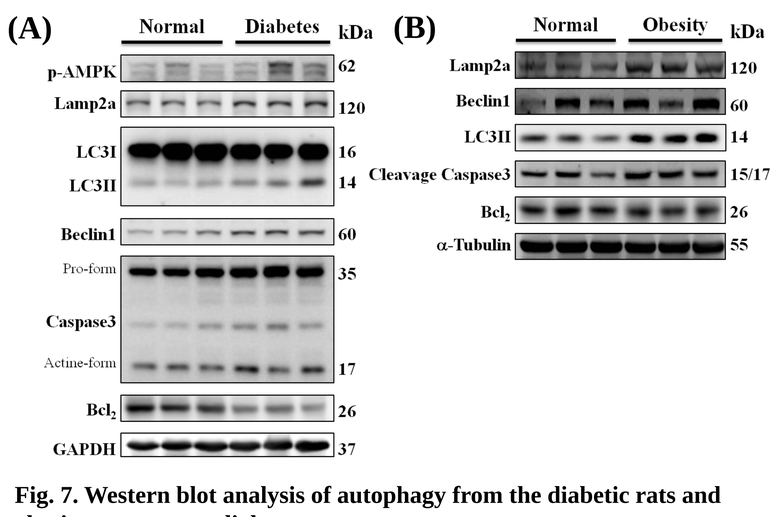

Fig. 7. Western blot analysis of autophagy from the diabetic rats and obesity rats myocardials.

The western blot was used to analyses (A) diabetes and (B) obesity rat myocardiam.

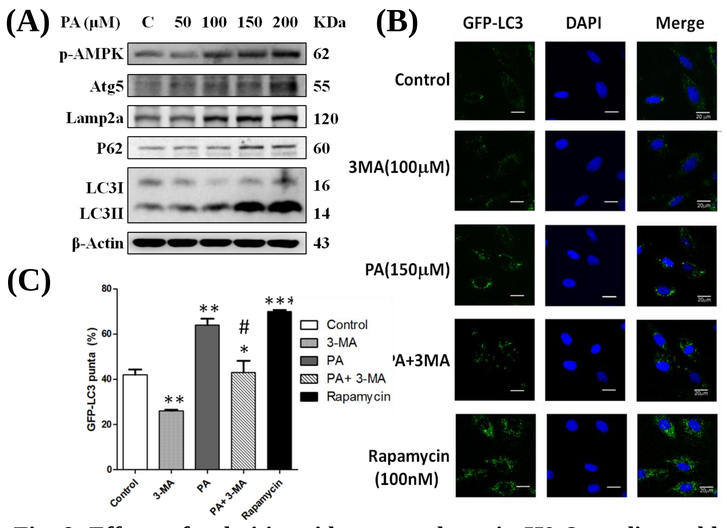

Fig. 8. Effects of palmitic acid on autophagy in H9c2 cardiomyoblast cells.

(A) H9c2 cells were treated with different dosages of palmitic acid (50μM、100 μM、150 μM、200 μM) for 24 hr. Western blot was used to analysis total cell lysates. (B, C) Puncta assay was performed after transfection with GFP-LC3 for 24 hr, H9c2 cells were treated with 150μM palmitic acid for 24 hr. Data are presented as the mean ± S.E.M. (n=3). ***P<0.001,P<0.01 and ,*P<0.1, represent significantly difference versus with control. #P<0.1, represent significantly difference versus with palmitic acid.**

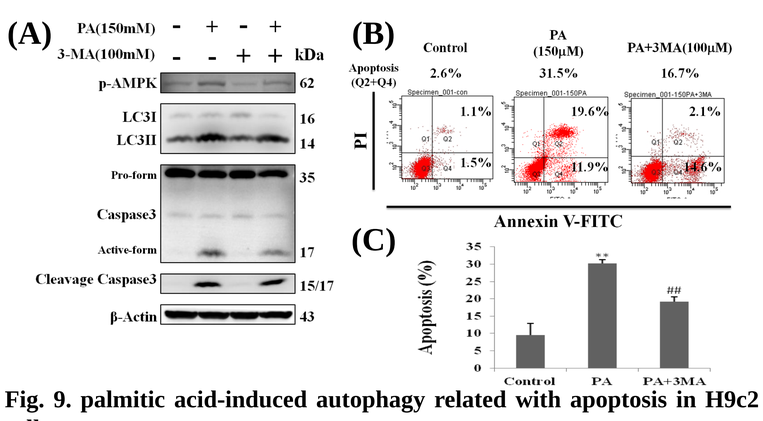

Fig. 9. palmitic acid-induced autophagy related with apoptosis in H9c2 cells.

(A) Western blot was used to analysis total cell lysates from H9c2 cells were treat with 100μM 3MA for 2hr before palmitic acid treated with 150μM for 24 hr. (B,C) H9c2 cells were treat with 100μM 3MA for 2hr before palmitic acid treated with 150μM for 24 hr by flow cytometry. Data are presented as the mean ± S.E.M. (n=3). **P<0.01, represent significantly difference versus control. ##P<0.01, represent significantly difference with that of palmitic acid treatment group.

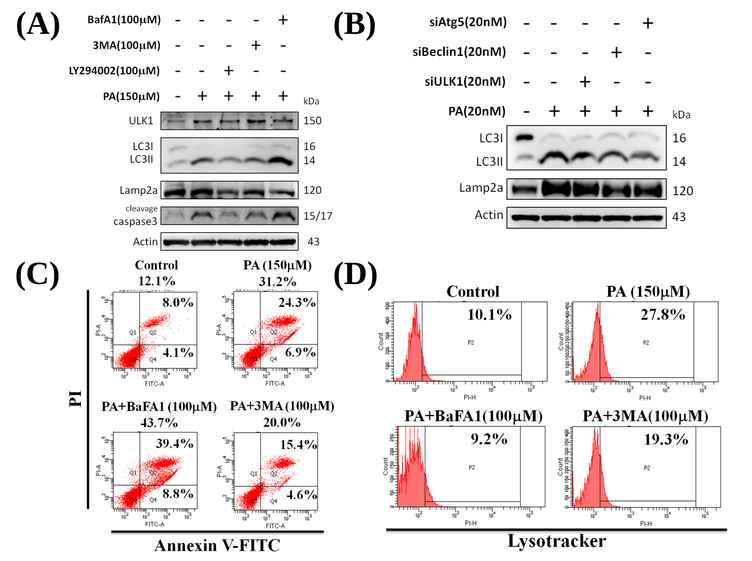

Fig. 10. Palmitic acid induced apoptosis is though autophagosome and not autolysosome formation in H9c2 cardiomyoblast cells.

After treated with (A) 100μM LY294002, 100μM 3-MA, 100μM Bafilomycin A1 for 1hr and (B) 20nM siRNA-ULK1, 20nM siRNA-Beclin1, 20nM siRNA-Atg5 for 24 hr, (C, D) BafA1 and 3MA for 1hr, H9c2 cells were treated with palmitic acid (150μM) for 24hr. Palmitic acid induced autophagy and apoptosis were measured by Western blot, Flow cytometry and Lysotracker.

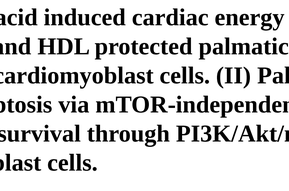

Fig. 11. The HDL protects mTOR-independent signaling pathway in palmitic acid-induced autophagy in H9c2 cardiomyoblast cells.

After treatement with different dosages of HDL for 24 hr, H9c2 cells were treated with 150μM palmitic acid for 24hr. HDL protects mTOR-independent signaling pathway in palmitic acid-induced autophagy and apoptosis were measured by Western blot.

Conclusion

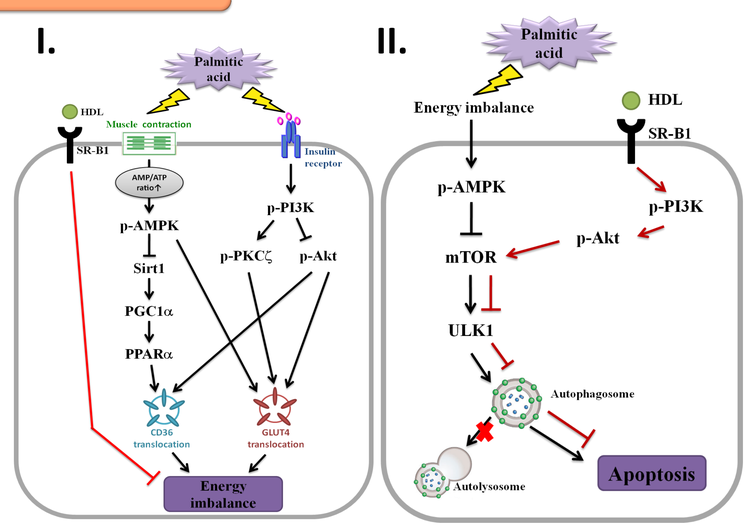

Fig. 12 (I) Palmitic acid induced cardiac energy imbalance by switching CD36 and GLUT4 and HDL protected palmatic acid-induced energy imbalance in H9c2 cardiomyoblast cells. (II) Palmitic acid induced autophagy and apoptosis via mTOR-independent signaling pathway, but HDL enhanced cell survival through PI3K/Akt/mTOR signaling pathway in H9c2 cardiomyoblast cells.