MS.ID-IJPBS-2548
International Journal of Pharmacy and Biological Sciences-IJPBS™ (2024) 14 (3): 01-07
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | OA Journal | MCI Approved | Index Copernicus

Differential Effects of Cholesterol and Palmitic Acid on Autophagy, Cell Death, and Metabolic Pathways in HepG2 Cells.

A. Shima and J. Kamishikiryo*
Department of Biochemistry, Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University, 985-1, Sanzo, Higashimura-Cho, Fukuyama, Hiroshima 729-0292, Japan.

Received: 12 Mar 2024 / Accepted: 8 Apr 2024 / Published online: 1 Jul 2024
***Corresponding Author Email:** j-kamishi@fukuyama-u.ac.jp

Abstract
The increase in high-calorie diets, notably those rich in animal fats, has contributed to an increased prevalence of obesity, metabolic syndrome, and related diseases. This study investigates the cellular effects of palmitic acid and cholesterol, which are significant components of animal fats, on HepG2 cells, focusing on autophagy, cell death, and key metabolic pathways. Our results demonstrate that treatment with 80 µM cholesterol resulted in significant intracellular cholesterol accumulation and a marked decrease in cell viability. Similarly, 800 µM palmitic acid increased lipid droplet formation and reduced the cell viability. Although cholesterol promoted autophagy and inhibited p70S6 kinase activity, it did not affect AMP-activated protein kinase (AMPK) or acetyl-CoA carboxylase (ACC) phosphorylation. Conversely, palmitic acid suppressed autophagy, inhibited p70S6 kinase activity, activated AMPK, and decreased ACC phosphorylation. Oleic acid, a monounsaturated fatty acid, increased lipid droplet size, but did not adversely affect cell viability or autophagy. These findings suggest that the cytotoxic effects of cholesterol and palmitic acid are mediated by distinct mechanisms involving autophagy regulation and metabolic pathway disruption. These insights will pave the way for future research further to elucidate lipid-specific pathways and their implications in metabolic health. Emphasizing the reduction in dietary palmitic acid and the inclusion of less harmful fatty acids holds promise for mitigating cholesterol toxicity and preventing lifestyle-related diseases.

Keywords
Autophagy, Cell death, Cholesterol, Fatty acid, Metabolic pathways

*****

1. INTRODUCTION:
Global increase in the consumption of high-calorie diets has led to an increase in the prevalence of various diseases [1,2]. Diets rich in animal fats are known to contribute to obesity and metabolic syndrome, thereby increasing the risk of lifestyle-related diseases such as dyslipidemia and atherosclerotic diseases [3]. According to previous studies, the average intake of dietary fats, including animal fats, has significantly increased over the past few decades, particularly in high- and upper-middle-income countries. This increase has been correlated with an increase in obesity and metabolic disorders worldwide, highlighting the impact of changing dietary patterns on public health [4,5]. Animal fats

are high in saturated fatty acids, such as palmitic acid and cholesterol.

Additionally, some animal fats contain monounsaturated fatty acids such as oleic acid. Saturated fatty acids, particularly palmitic acid, have been shown to contribute to insulin resistance and inflammation, which are critical factors in the development of metabolic syndromes and cardiovascular diseases [6]. The impact of cholesterol on health is also significant, as cholesterol is a major component of atherosclerotic plaques that lead to coronary artery disease [7].

Palmitic acid promotes inflammatory responses and inhibits autophagy, leading to cell death when present in excess [8–10]. Moreover, excessive cholesterol has been linked to an increased risk of steatotic liver disease, cirrhosis, and metabolic disorders such as atherosclerosis and metabolic syndrome [11–13]. Studies have also indicated that palmitic acid and cholesterol affect key metabolic signaling pathways. For instance, palmitic acid has been shown to inhibit cellular energy homeostasis [14–16]. Cholesterol accumulation does not significantly affect cellular energy homeostasis but alters lipid metabolism [17,18]. Furthermore, palmitic acid and cholesterol have been shown to suppress protein synthesis and cell growth. Suppression of protein synthesis and impaired cell growth contribute to cellular dysfunction and increased susceptibility to metabolic stress.

While it is well established that an increased intake of animal fats is associated with a higher incidence of cardiovascular diseases and cancer, the specific effects of individual lipid components at the cellular level remain inconsistent. In particular, the precise effects of palmitic acid and cholesterol on the metabolic pathways involving p70S6 kinase, AMP-activated protein kinase (AMPK), and acetyl-CoA carboxylase (ACC) remain unclear. p70S6 kinase is crucial for protein synthesis and cell growth, as it is involved in the translation of proteins necessary for cell cycle progression. AMPK is a central regulator of cellular energy homeostasis and functions as an energy sensor that activates pathways that generate ATP, while inhibiting those that consume ATP. ACC, a key enzyme in fatty acid synthesis, converts acetyl-CoA to malonyl-CoA, which is the first step in this biosynthetic pathway. The inhibition of ACC phosphorylation disrupts lipid metabolism, leading to altered lipid homeostasis. Although palmitic acid can inhibit AMPK phosphorylation, and cholesterol can affect ACC phosphorylation, the detailed mechanisms through which these lipids influence these key metabolic regulators and their downstream effects on cellular metabolism remain unclear. Further research is necessary to elucidate the interactions between these lipids and their metabolic pathways at the molecular level and to understand their implications for metabolic health and disease.

In this study, we investigated the effects of palmitic acid and cholesterol, components of animal fats, on HepG2 cells, focusing on autophagy, cell death, and metabolic pathways.

2. MATERIALS AND METHODS:

2-1. materials

A solution of 30 w/v % albumin solution (fatty acid-free), Oil Red O, methyl-β-cyclodextrin, chloroquine diphosphate, and Immunostar Zeta chemiluminescence reagents were purchased from FUJIFILM Wako Chemicals (Osaka, Japan). Sodium palmitate was purchased from the Tokyo Chemical Industry (Tokyo, Japan). Sodium oleate was purchased from Nacalai Tesque Inc. (Kyoto, Japan). ProLong Diamond Antifade Mountant and a complete protease inhibitor cocktail were obtained from ThermoFisher Scientific (Waltham, MA, USA) and Sigma-Aldrich (St. Louis, MO, USA), respectively. The Cell Counting Kit-8 was purchased from Dojindo (Kumamoto, Japan). Anti-LC3 (Cat. No. PM036) and anti-b-Actin (Cat. No. M177-3) antibodies were purchased from MBL (Nagoya, Japan). Anti-AMPKα (Cat. No. 5831), anti-Phosphor-AMPKα (Thr172) (Cat. No. 2535), anti-Acetyl-CoA Carboxylase (Cat. No. 3676), anti-Phospho-Acetyl CoA Carboxylase (Ser79) (Cat. No. 11818), anti-p70 S6 Kinase (Cat. No. 2708), and anti-Phospho-p70 S6 Kinase (Cat. No. 9234) antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). The horseradish peroxidase-conjugated goat anti-rabbit IgG (H+L) polyclonal antibody (Cat. No. 611-1302) was purchased from Rockland Immunochemical (Gilbertsville, PA, USA). The horseradish peroxidase-conjugated goat anti-mouse IgG (H+L) polyclonal antibody (Cat. No. A90-116P) was purchased from Bethyl (Montgomery, TX, USA). The human hepatoma cell line, HepG2 (RCB1648), was provided by the RIKEN BioResource Research Center through the National Bioresource Project of the Japan Agency for Medical Resource and Development.

2-2. cell culture

HepG2 cells were cultured in Medium A (Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 units/mL penicillin, 100 µg/mL streptomycin, and non-essential amino acids) containing 10% fetal bovine serum (FBS) at 37 ℃ in 5% CO2. Medium A supplemented with 37.5 µM cholesterol/methyl-β-cyclodextrin was used for cholesterol-adding treatment, and medium A

containing fatty acid-BSA conjugates was used for fatty acid-adding treatment.

2-3. cell viability assay

Cell viability was measured using a Cell Counting Kit-8 (CCK-8) according to the manufacturer's instructions. Briefly, the HepG2 cells were seeded at a density of 6×10^3 cells/well in 96-well plates. The next day, the cells were treated with fatty acids or cholesterol in medium A for 12 h. After the treatment, CCK-8 solution was added to the medium before further incubation for 3 h at 37 ℃ in 5% CO2. After incubation, the absorbance was measured at 450 nm.

2-4. western blotting

HepG2 cells were seeded at a density of 3×10^5 cells/well in 12-well plates. The next day, the cells were treated with fatty acids or cholesterol in medium A for 12 h. After the treatment, cells were washed with cold phosphate-buffered saline (PBS) and lysed with lysis buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, 0.02% bromophenol blue, 1 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 2 mM Na_3VO_4, 2 mM NaF, 1 mM sodium molybdate, complete protease inhibitor cocktail) and transferred to microcentrifuge tubes. Cell lysates were then sonicated and boiled for 5 min. The lysates were subjected to SDS-PAGE, and the proteins were transferred to a polyvinylidene membrane. The membranes were blocked with TBST (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% Tween 20) containing 1% skim milk. The membrane was probed with primary antibody overnight at 4°C and incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. Signals were visualized using the Immunostar Zeta chemiluminescence reagents. The signal intensities were analyzed using a LuminoGraph II imaging analyzer (ATTO, Tokyo, Japan).

2-5. confocal imaging

HepG2 cells were seeded on a cover glass and treated with fatty acids or cholesterol for 12 h. Cells were washed in PBS and fixed with 4% paraformaldehyde. They were then stained with 50 µg/mL Filipin solution in the dark for 30 min for cholesterol staining and identification. Cells were stained with Oil Red O solution for 20 min for lipid droplet staining. The cells were then washed with PBS and mounted using ProLong Diamond Antifade Mountant. Confocal images were obtained using a laser-scanning microscope (Leica DM2500).

2-6. statistical analysis

Data are expressed as mean ± standard deviation (S.D.). Statistical analyses were performed using a one-way analysis of variance followed by Tukey's post-hoc test. The sample sizes are shown in the figure legends. Statistical significance was set at $P < 0.05$.

3. RESULTS:

3-1. effects of fatty acids and cholesterol on HepG2 cell lipid accumulation

We first investigated whether lipid accumulation occurred in HepG2 cells after treatment with cholesterol, palmitic acid, or oleic acid (Figure 1a). Intracellular cholesterol was detected using filipin, a fluorescent dye that binds to cholesterol. When the HepG2 cells were treated with various cholesterol concentrations for 12 h, no significant cholesterol accumulation was observed at low concentrations. However, treatment with 80 µM cholesterol resulted in approximately 30-fold more significant cholesterol accumulation compared with the control group (Figure 1b).

Lipid accumulation in HepG2 cells treated with fatty acids was detected by Oil Red O staining (Figure 1a). Treatment of HepG2 cells with palmitic acid or oleic acid resulted in a significant increase in intracellular lipid droplets at 400 µM and 800 µM concentrations (Figure 1c). Notably, treatment with palmitic acid did not alter the size of lipid droplets. In contrast, treatment with oleic acid at concentrations of 400 µM and 800 µM led to a significant increase in lipid droplet size, with the droplets becoming more than eight times more significant compared with the untreated group (Figure 1d).

3-2. effects of fatty acids and cholesterol on HepG2 cell viability

We investigated the effects of cholesterol, palmitic acid, and oleic acid on HepG2 cell viability (Figure 2). HepG2 cells were treated with varying concentrations of cholesterol, palmitic acid, and oleic acid for 12 h, and cell viability was assessed using the CCK-8 assay. The results indicated that cholesterol significantly reduced cell viability at a concentration of 80 µM, decreasing it to 47% compared with the untreated group. In the case of palmitic acid treatment, a significant reduction in cell viability was observed at a concentration of 800 µM, decreasing it to 25% compared with the untreated group. Conversely, treatment with oleic acid did not reduce cell viability; instead, a significant increase in viability was observed at a concentration of 800 µM.

3-3. palmitic acid and cholesterol affect the expression level of microtubule-associated protein light chain 3-II (LC3B-II) protein

To investigate the effects of cholesterol, palmitic acid, and oleic acid on autophagy in HepG2 cells, we examined LC3B-II levels after lipid addition by western blotting (Figure 3). LC3B-II protein is an autophagy marker; during autophagosome

formation, phosphatidylethanolamine is conjugated to LC3B-I, producing LC3B-II. When HepG2 cells were treated with cholesterol and palmitic acid, the amount of LC3B-II increased compared with that in the control group. In contrast, treatment with oleic acid resulted in little change in LC3B-II protein levels compared with the control.

To determine whether cholesterol and palmitic acid promote or inhibit autophagy, we examined LC3B-II protein levels in chloroquine. LC3B-II protein levels can increase because of enhanced initiation of autophagy or inhibition of lysosomal degradation. Chloroquine inhibits lysosomal degradation by preventing lysosomal acidification. Therefore, an increase in LC3B-II protein levels in the presence of chloroquine indicates enhanced autophagy, whereas a small change suggests the inhibition of autophagy. Upon examining the effects of cholesterol and palmitic acid in the presence of 20 µM chloroquine, we observed a substantial increase in LC3B-II protein levels with cholesterol treatment. In contrast, palmitic acid treatment resulted in little change in LC3B-II protein levels, even with chloroquine.

3-4. palmitic acid and cholesterol suppress the phosphorylation of p70S6 kinase

To investigate the effects of cholesterol, palmitic acid, and oleic acid on cellular protein synthesis and growth, we examined the phosphorylation status of the p70S6 kinase (Figure 4a). p70S6 kinase is a critical signaling molecule involved in protein synthesis and cell growth, activating ribosomal function through the phosphorylation of ribosomal protein S6, thereby promoting the synthesis of proteins essential for cellular growth and proliferation. HepG2 cells treated with cholesterol and palmitic acid exhibited reduced phosphorylation of p70S6 kinase compared with the control cells.

3-5. palmitic acid and cholesterol affect the phosphorylation level of metabolic pathway proteins

To investigate the effects of cholesterol, palmitic acid, and oleic acid on metabolic pathways, we examined the phosphorylation statuses of AMPK and ACC (Figure 4b), which play crucial roles in cellular energy and lipid metabolism. AMPK is activated when intracellular ATP levels decrease and AMP levels increase, leading to the inhibition of fatty acid and protein synthesis. ACC is a rate-limiting enzyme in fatty acid synthesis, and its activity is suppressed when phosphorylated by AMPK, which inhibits cellular fatty acid synthesis. In HepG2 cells treated with cholesterol or oleic acid, no significant changes in the phosphorylation states of AMPK and ACC were observed compared with those in the control. However, in cells treated with palmitic acid, there was an increase in AMPK phosphorylation and a decrease in ACC phosphorylation compared with those in the control.

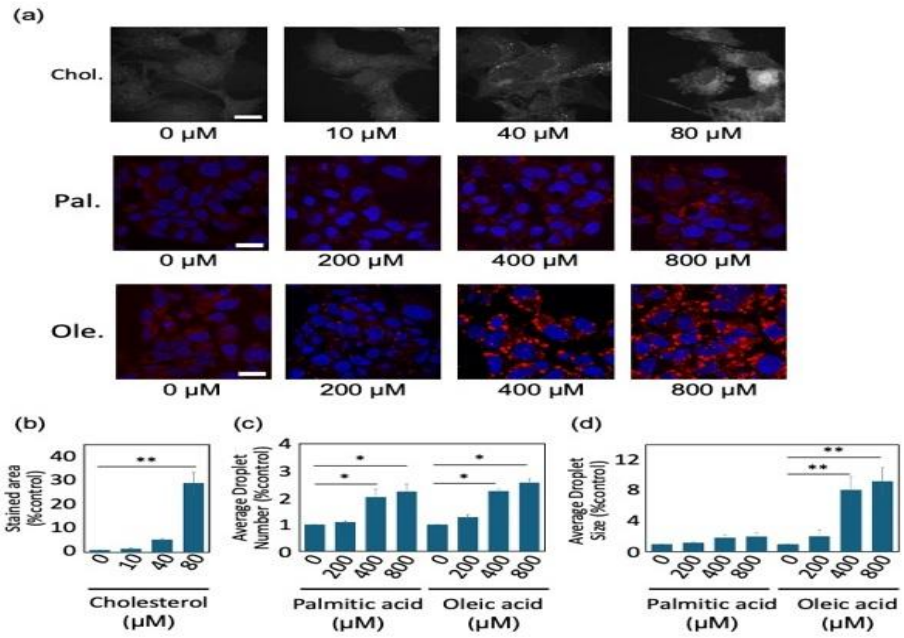

Figure 1: Effects of cholesterol and fatty acids on HepG2 cell lipid accumulation

(a) Confocal microscopic images of intracellular cholesterol and lipid droplets. Scale bar = 20 µm. (b) Quantification of cholesterol-stained areas in HepG2 cells. (c) Quantification of the average number of lipid droplets in HepG2 cells. (d) Quantification of the average size of lipid droplets in HepG2 cells. Significant differences compared to the control cells are shown as *p < 0.05, **p < 0.01, using Tukey's post-hoc test.

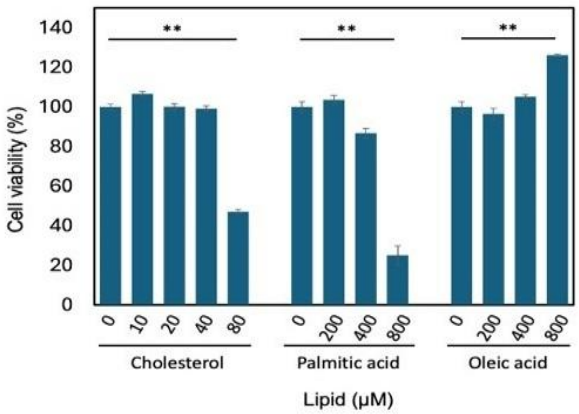

Figure 2: HepG2 cell viability after treatment with the indicated concentrations of lipids.
Cell viability was calculated as a percentage of non-treated cells. Data are expressed as the mean ± standard deviation (n =4). Significant differences compared with the non-treated cells are shown *p < 0.05 or **p < 0.01 using Tukey's post-hoc test.

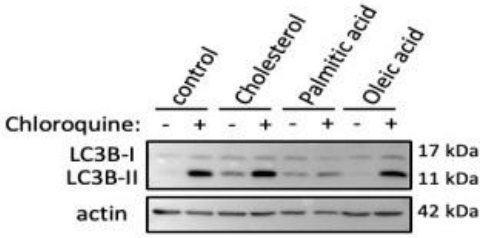

Figure 3: Expression levels of LC3B-II protein by western blotting.

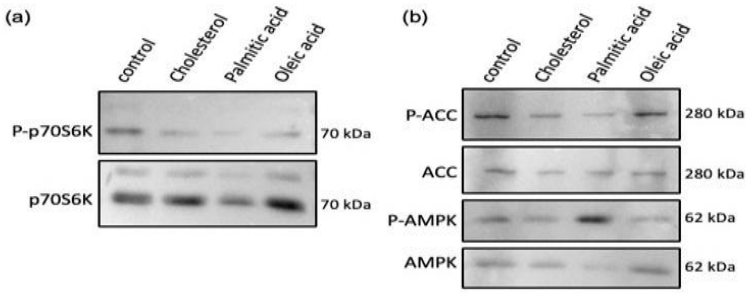

Figure 4: Expression levels of protein synthesis and metabolic pathway proteins by western blotting.
(a) Expression levels of p70S6 kinase. (b) Expression levels of AMPK and ACC.

4. DISCUSSION:

In the present study, we investigated the effects of cholesterol and palmitic acid on autophagy, cell death, and metabolic pathways in HepG2 cells. When HepG2 cells were treated with 80 µM cholesterol, a significant accumulation of cholesterol within the cells was observed, resulting in a marked decrease in cell viability. Similarly, treatment with 800 µM palmitic acid led to increased intracellular lipid droplets and a significant reduction in cell viability. Although both cholesterol and palmitic acid substantially decreased cell viability, their effects on autophagy, protein synthesis, and metabolic pathways differed. Cholesterol promotes autophagy and inhibits the activity of p70S6 kinase, which is involved in protein synthesis but does not affect

AMPK or ACC activity. In contrast, palmitic acid suppresses autophagy, activates AMPK, and inhibits ACC and p70S6 kinase activity.

We examined the effects of oleic acid, a monounsaturated fatty acid, on HepG2 cells. Oleic acid induced a significant accumulation of lipid droplets within the cells but only slightly increased cell viability without affecting autophagy, p70S6 kinase activity, or metabolic pathways. Therefore, an increase in intracellular lipid droplets does not directly lead to decreased cell viability; instead, the disruption of protein synthesis and energy supply contributes to reduced cell viability.

Previous studies have confirmed that progression of metabolic dysfunction-associated steatohepatitis is associated with the autophagy suppression [19–21]. When autophagy is inhibited, cellular waste and damage accumulation reduces the ability of the cell to respond to stress, leading to cell death [22]. However, excessive autophagy degrades essential cellular components, also resulting in decreased cell viability [23,24]. The p70S6 kinase regulates the synthesis of various proteins necessary for cell survival, and both cholesterol and palmitic acid inhibit its activity. Decreased protein synthesis may weaken the adaptive capacity of cells under stressfull conditions and promote cell death. AMPK is activated when cells experience low-energy conditions or nutritional stress. In energy-deficient cells, AMPK activation inhibits mTORC1 activity and induces autophagy. Palmitic acid has been reported to increase the levels of Rubicon, an autophagy-inhibitory protein [25–27]. Thus, even under low-energy or nutritional stress conditions, where AMPK is activated, increased levels of Rubicon may inhibit autophagy, prevent stress alleviation, and lead to cell death.

A limitation of our study is that we used only HepG2 cells; therefore, caution is needed when extrapolating these results to other cell types, particularly hepatocytes. However, our findings are noteworthy considering that animal fats rich in cholesterol and palmitic acid can contribute to diseases in various organs, including the liver. Our study confirmed that cholesterol and palmitic acid induce cell death in HepG2 cells, but the underlying cellular phenomena differ significantly. Previously, we reported that long-chain fatty acids exhibit strong cytotoxicity, whereas short- and medium-chain fatty acids show little to no cytotoxicity [28]. We propose that reducing the amount of palmitic acid in food and replacing it with short- and medium-chain fatty acids can mitigate cholesterol toxicity and prevent lifestyle-related diseases.

5. CONCLUSIONS:

This study demonstrated that cholesterol and palmitic acid induce significant cell death in HepG2 cells, albeit through distinct mechanisms. Cholesterol promoted autophagy and inhibited protein synthesis without affecting AMPK or ACC activity. In contrast, palmitic acid suppresses autophagy, inhibits protein synthesis, activates AMPK, and inhibits ACC, leading to cell death. This highlights that the disruption of protein synthesis and energy supply are critical factors in the reduction of cell viability.

6. ACKNOWLEDGEMENT:

We would like to thank Editage (www.editage.jp) for English language editing.

7. CONFLICT OF INTEREST:

There are no conflicts of interest to declare.

8. REFERENCES

[1] Friedman JM. Obesity in the new millennium. Nature. 2000;404: 632–634.
[2] Calle EE, Thun MJ, Petrelli JM, Rodriguez C, Heath CW Jr. Body-mass index and mortality in a prospective cohort of U.S. adults. N Engl J Med. 1999;341: 1097–1105.
[3] Schaffer JE. Lipotoxicity: when tissues overeat. Curr Opin Lipidol. 2003;14: 281–287.
[4] Forouhi NG, Krauss RM, Taubes G, Willett W. Dietary fat and cardiometabolic health: evidence, controversies, and consensus for guidance. BMJ. 2018;361. doi:10.1136/bmj.k2139
[5] Meijaard E, Abrams JF, Slavin JL, Sheil D. Dietary Fats, Human Nutrition and the Environment: Balance and Sustainability. Front Nutr. 2022;9: 878644.
[6] Leamy AK, Egnatchik RA, Young JD. Molecular mechanisms and the role of saturated fatty acids in the progression of non-alcoholic fatty liver disease. Prog Lipid Res. 2013;52: 165–174.
[7] Tabas I. Consequences of cellular cholesterol accumulation: basic concepts and physiological implications. J Clin Invest. 2002;110: 905–911.
[8] Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest. 2006;116: 3015–3025.
[9] Holland WL, Bikman BT, Wang L-P, Yuguang G, Sargent KM, Bulchand S, et al. Lipid-induced insulin resistance mediated by the proinflammatory receptor TLR4 requires saturated fatty acid-induced ceramide biosynthesis in mice. J Clin Invest. 2011;121: 1858–1870.
[10] Pal D, Dasgupta S, Kundu R, Maitra S, Das G, Mukhopadhyay S, et al. Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. Nat Med. 2012;18: 1279–1285.
[11] Jiang Y, Wu L, Zhu X, Bian H, Gao X, Xia M. Advances in management of metabolic dysfunction-associated

steatotic liver disease: from mechanisms to therapeutics. Lipids Health Dis. 2024;23: 95.

[12] Del Villar-Carrero RS, Blanco A, Ruiz LD, García-Blanco MJ, Segovia RC, de la Garza RG, et al. Bridging Metabolic-Associated Steatotic Liver Disease and Cardiovascular Risk: A Potential Role for Ketogenesis. Biomedicines. 2024;12. doi:10.3390/biomedicines12030692

[13] Branković M, Dukić M, Gmizić T, Popadić V, Nikolić N, Sekulić A, et al. New Therapeutic Approaches for the Treatment of Patients with Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) and Increased Cardiovascular Risk. Diagnostics (Basel). 2024;14. doi:10.3390/diagnostics14020229

[14] Xu X, Luo D, Xuan Q, Lu P, Yu C, Guan Q. Atlas of metabolism reveals palmitic acid results in mitochondrial dysfunction and cell apoptosis by inhibiting fatty acid β-oxidation in Sertoli cells. Front Endocrinol. 2022;13: 1021263.

[15] Raja AA, Dandare A, Khan MJ, Khan MJ. Free Fatty Acid Overload Targets Mitochondria: Gene Expression Analysis of Palmitic Acid-Treated Endothelial Cells. Genes. 2022;13. doi:10.3390/genes13101704

[16] Ma K, Sheng W, Gao R, Feng J, Huang W, Cui L, et al. Ethanolic extract of root from Arctium lappa L ameliorates obesity and hepatic steatosis in rats by regulating the AMPK/ACC/CPT-1 pathway. J Food Biochem. 2022;46: e14455.

[17] Li M, Wang Z, Wang P, Li H, Yang L. TFEB: A Emerging Regulator in Lipid Homeostasis for Atherosclerosis. Front Physiol. 2021;12: 639920.

[18] Luo J, Yang H, Song B-L. Mechanisms and regulation of cholesterol homeostasis. Nat Rev Mol Cell Biol. 2020;21: 225–245.

[19] Chavez-Tapia NC, Rosso N, Tiribelli C. Effect of intracellular lipid accumulation in a new model of non-alcoholic fatty liver disease. BMC Gastroenterol. 2012;12: 20.

[20] Czaja MJ, Ding W-X, Donohue TM Jr, Friedman SL, Kim J-S, Komatsu M, et al. Functions of autophagy in normal and diseased liver. Autophagy. 2013;9: 1131–1158.

[21] González-Rodríguez A, Mayoral R, Agra N, Valdecantos MP, Pardo V, Miquilena-Colina ME, et al. Impaired autophagic flux is associated with increased endoplasmic reticulum stress during the development of NAFLD. Cell Death Dis. 2014;5: e1179.

[22] Yang L, Li P, Fu S, Calay ES, Hotamisligil GS. Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. Cell Metab. 2010;11: 467–478.

[23] Qin P, Li Q, Zu Q, Dong R, Qi Y. Natural products targeting autophagy and apoptosis in NSCLC: a novel therapeutic strategy. Front Oncol. 2024;14: 1379698.

[24] Kim A, Im M, Yim N-H, Kim T, Ma JY. A novel herbal medicine, KIOM-C, induces autophagic and apoptotic cell death mediated by activation of JNK and reactive oxygen species in HT1080 human fibrosarcoma cells. PLoS One. 2014;9: e98703.

[25] Tanaka S, Hikita H, Tatsumi T, Sakamori R, Nozaki Y, Sakane S, et al. Rubicon inhibits autophagy and accelerates hepatocyte apoptosis and lipid accumulation in nonalcoholic fatty liver disease in mice. Hepatology. 2016;64: 1994–2014.

[26] Zhong Y, Wang QJ, Li X, Yan Y, Backer JM, Chait BT, et al. Distinct regulation of autophagic activity by Atg14L and Rubicon associated with Beclin 1-phosphatidylinositol-3-kinase complex. Nat Cell Biol. 2009;11: 468–476.

[27] Matsunaga K, Saitoh T, Tabata K, Omori H, Satoh T, Kurotori N, et al. Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. Nat Cell Biol. 2009;11: 385–396.

[28] J. Kamishikiryo and A. Shima. Differential Effects of 2 to16 Carbon Saturated Fatty Acids on Autophagy and Cell Viability of HepG2 Cells. IJPBS. 2023;13: 223-230. doi:10.5281/zenodo.10608294