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Study of Epicardial Fat Lipid in Coronary Heart Disease on the Regulation Mechanism of Metabolism

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Objective: Coronary heart disease (CHD), emerging as a common chronic disease, is threatening the lives of middle-aged and older people in China, with a gradual increase in morbidity and mortality. However, the metabolic mechanism of CHD remains unclear, necessitating a comprehensive elucidation. Therefore, this study aimed to examine the underlying regulatory mechanism of epicardial lipid metabolism in CHD.

Methods: This study collected epicardial adipose tissue samples (n = 40), including 20 patients with CHD and 20 non-CHD. 3T3L1 was induced to differentiate into mature adipocytes *in vitro* and subsequently treated with different concentrations of oxidized low-density lipoprotein (ox-LDL), glucose, and lipopolysaccharide (LPS). The contents of glycerol and triglyceride were determined using corresponding kits. Moreover, the concentrations of cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) were assessed utilizing ELISA. Furthermore, western blotting analysis and qRT-PCR were employed to determine protein and mRNA expression levels. Additionally, immunofluorescence analysis was utilized to assess the expression of lipid droplet coating protein perilipin A. The morphology and count of lipid droplets were observed using a confocal microscope.

Results: Compared to the non-CHD group, the level of triglyceride and perilipin A increased significantly, while the content of glycerol, PKA, cAMP, adipose triglyceride lipase (ATGL), and hormone-sensitive lipase (HSL) decreased in the CHD group. Furthermore, high ox-LDL and glucose significantly decreased small lipid droplets, glycerol, ATGL, and HSL while substantially increasing large lipid droplets, triglyceride, cAMP, PKA, and perilipin A in 3T3L1 cells. Additionally, high LPS concentration significantly increased small lipid droplets, glycerol, cAMP, PKA, ATGL, and HSL, and decreased large lipid droplets, triglyceride, and perilipin A.

Conclusions: In summary, high ox-LDL and glucose levels regulate the lipolysis of 3T3L1 adipocytes by regulating the cAMP-PKA and perilipin A-ATGL-HSL pathways. However, high LPS can promote the hydrolysis of 3T3L1 adipocytes.

Keywords: coronary heart disease; epicardial fat; perilipin A; ATGL-HSL pathway; cAMP-PKA pathway

Introduction

As scientific and technological advancements in China continue, the improvement of people's living standards has prompted cardiovascular diseases, particularly coronary heart disease (CHD). This condition emerges as the most common chronic disease threatening the lives of middle-aged and older people worldwide [1]. The pathological changes of CHD primarily involve the deposition of atherosclerotic plaque on the coronary artery wall, leading to lumen stenosis [2]. As the disease progresses, the original plaque may rupture, resulting in bleeding or lipid accumulation, further narrowing the coronary artery and leading to prolonged ischemia and hypoxia in the corresponding stage of the myocardium. Eventually, this process leads to myocardial infarction, posing a severe threat to human life and health [3]. There are many risk factors for CHD, among which obesity and its associated metabolic diseases are the most critical factors. Indeed, obesity stands as a significant risk factor in the occurrence and progression of CHD [4]. Compared to non-obese patients, obese individuals with higher visceral fat levels exhibit faster development of CHD, worse prognosis, and higher mortality rates. Epicardial fat (EAT) holds significant importance among visceral adipose tissues and contributes significantly to the occurrence and development of CHD [5,6]. However, the precise mechanism underlying this association remains unclear.

The hydrolysis of fat cells generally involves three sequential steps. The first and most crucial step is mediated by adipose triglyceride lipase (ATGL), an enzyme known as triacylglycerol lipase. The second step is predominantly conducted by hormone-sensitive lipase (HSL), a hormone-sensitive lipase, and the third and final step is mediated by MGL, a monoglyceride lipase [7]. Through the collaborative action of these three crucial enzymes, triglycerides are

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gradually degraded into glycerol and three molecules of free fatty acids [8]. Under normal physiological conditions, perilipin A and CGI-58 are closely correlated and positioned on the surface of lipid droplets. However, upon activating the B adrenoceptor, perilipin A is phosphorylated through the cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA) pathway. Subsequently, perilipin A dissociates from CGI-58, resulting in activation of CGI-58. The activated CGI-58 interacts with ATGL, significantly improving its activity and accelerating fat catabolism. Furthermore, a theory of the fat-vascular regulation axis was proposed by a Japanese researcher: fat factors secreted by adipocytes can directly influence vascular endothelial cells and smooth muscle cells, thereby affecting their functional changes and consequently impacting arteriosclerosis, thrombosis, and inflammatory responses [9]. Therefore, visceral adipose tissue, especially EAT, correlates closely with CHD.

Researcher has suggested that lipopolysaccharides can activate inflammatory pathways and induce the release of several inflammatory factors, playing an essential role in the progression of atherosclerosis [10]. Moreover, abnormal oxidized low-density lipoprotein within the intima of blood vessels can lead to the rupture of unstable atherosclerotic plaque, eventually posing threats to human health [11]. Additionally, vascular dysfunction resulting from glucose abnormalities, particularly prevalent in diabetes patients, is closely linked to aggravated atherosclerosis and elevated myocardial infarction risk [12]. Therefore, investigating the underlying regulatory mechanism of epicardial lipid metabolism in CHD holds immense physiological significance and warrants further study. However, there has been limited investigation into the regulatory mechanism mediating epicardial lipid metabolism in CHD. Therefore, understanding how it affects EAT metabolism needs a thorough study. Hence, this study aimed to investigate the mechanisms underlying the lipocatabolism pathway in EAT of both CHD and non-CHD individuals. Additionally, we aimed to elucidate the impact of varying concentrations of glucose, lipopolysaccharide (LPS), and oxidized low-density lipoprotein (ox-LDL) on the hydrolysis pathway mechanism of 3T3L1 adipocytes. This study sought to establish a theoretical foundation for identifying the potential inhibitory effects of these pathways and their effective roles in preventing and treating obesity and CHD.

Materials and Methods

Collection of Tissue Specimens

This study included epicardial adipose tissue samples from 20 patients with CHD and 20 without CHD who underwent thoracotomy (non-CHD) between December 2021 and December 2022. The inclusion and exclusion criteria of the study participants were set as per [13]. The patients meeting the diagnostic criteria of CHD outlined in the Joint Report of the International Society of Cardiology and Association/World Health Organization Clinical Nomenclature Standardization Task Group, those having no severe diseases of liver, kidney, and other organ impairment and, patients with no history of mental illness were enrolled in this study. Exclusion criteria consisted of (1) patients with a medical history including congenital heart disease, cardiomyopathy, and other relevant diseases, (2) patients presented with diseases such as pericardial tamponade and abnormal sternum, which could affect the ultrasound measurement of EAT, (3) and those presented with cardiac dysfunction diseases such as arrhythmia and organic valvular heart disease. The CHD group of patients included eight males and twelve females with an average age of 58.32 ± 7.23.

Moreover, the non-CHD group consisted of nine males and eleven females with an average age of 55.21 ± 7.17. After collection, the tissue samples were washed with PBS to remove blood stains on the surface and stored at –80 °C for subsequent analysis. Approval for this study was obtained from the Medical Ethics Committee of Union Hospital Tongji Medical College, Huazhong University of Science and Technology (2018-S007). The study protocol adhered to the guidelines of the Declaration of Helsinki. The clinical information of the patients is shown in **Supplementary Table 1**.

Cell Culture and Treatment

The 3T3L1 preadipocytes were purchased from the American Type Culture Collection (FS-0079, Manassas, VA, USA). The cells were cultured in a DMEM medium (11965092, Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% FBS (10091148, Thermo Fisher Scientific, Inc., Waltham, MA, USA) and incubated in a 5% CO_2 environment at 37 °C. The cells underwent STR identification and mycoplasma testing. Once the cells reached full confluence, they were digested and then evenly inoculated into 6-well plates at a density of 1×10^5 cells per well. After achieving 90% confluence, the induction medium (bovine insulin 1 mg/L, 1- methyl-3-isobutyl xanthine 0.5 mmol/L, dexamethasone 1.0 μmol/L) was added and incubated for 2 days. After this, the induction medium was replaced with a modified medium containing only bovine insulin (1 mg/L) and continued for an additional 2 days of incubation. On the fourth day, the medium was changed to an ordinary basal medium. On the 8th day, cells were treated with varying concentrations of ox-LDL, glucose and LPS. Based on the concentration of ox-LDL, the cells were divided into three groups as follows: the low-ox-LDL group (25 μg/mL ox-LDL), the medium-ox-LDL group (50 μg/mL ox-LDL), the high-ox-LDL group (100 μg/mL ox-LDL) [14]. Similarly, based on glucose levels, the cells were categorized as the ow-glucose group (0 mmol/L glucose), the medium-glucose group (5.5 mmol/L glucose), and the high-glucose group (25 mmol/L glucose) [15]. Moreover, for LPS exposure, three groups of the

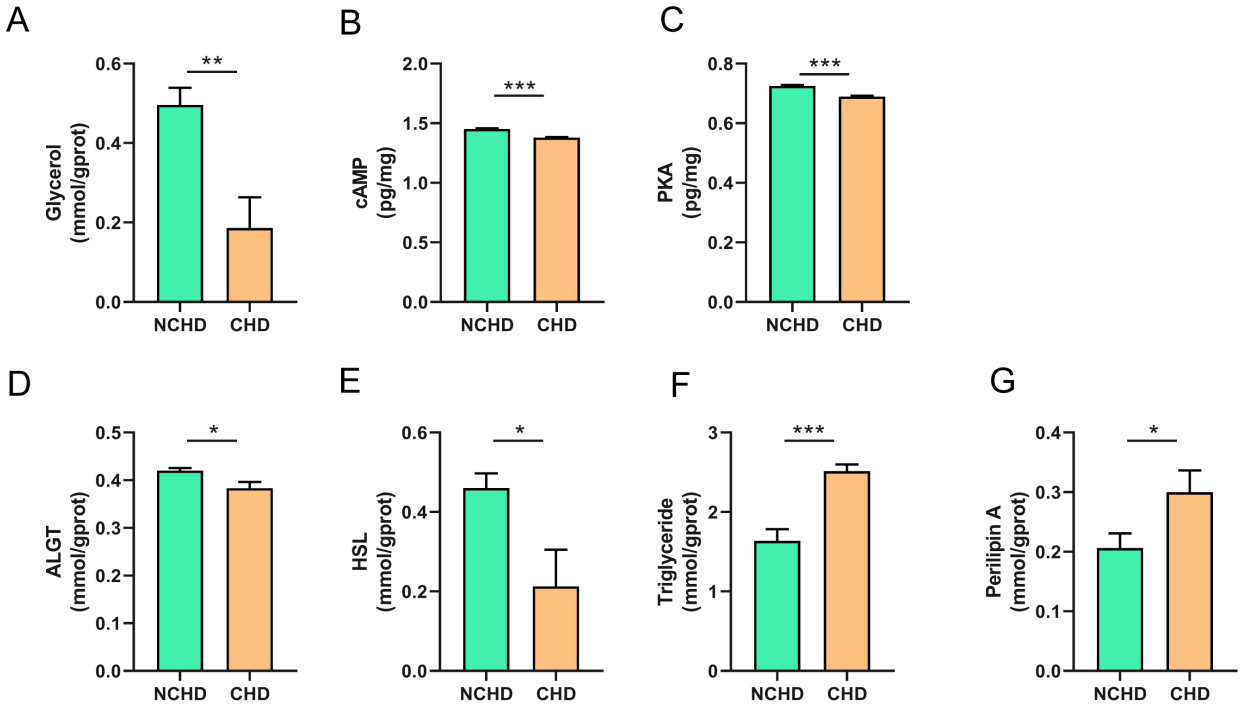

Fig. 1. Expression levels of catabolism-related indexes of triglycerides in epicardial fat in both the non-coronary heart disease (CHD) and CHD groups. The levels of glycerol (A), cyclic adenosine monophosphate (cAMP) (B), protein kinase A (PKA) (C), adipose triglyceride lipase (ATGL) (D), hormone-sensitive lipase (HSL) (E), triglyceride (F), and perilipin A (G) in epicardial fat of both the CHD and non-CHD groups were assessed using corresponding biochemical kits (n = 20). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

cells were as follows: the low-LPS group (25 ng/mL LPS), the medium-LPS group (50 ng/mL LPS), and the high-LPS group (100 ng/mL LPS) [16].

The Assay of Glycerol, Triglyceride, cAMP, and PKA Levels

The levels of glycerol (G0912F, Grace Biotechnology, Suzhou, China), triglyceride (G0910F, Grace Biotechnology, Suzhou, China), cAMP (ab290713, Abcam, Cambridge, UK), and PKA (ab139435, Abcam, Cambridge, UK) were evaluated utilizing corresponding detection kits. After a 2-day maturation induction with different concentrations of ox-LDL, LPS, and glucose, 0.1 mL of lysis solution was added to each well containing 1×10^5 cells. Subsequently, the levels of glycerol, triglyceride, cAMP, and PKA in 3T3L1 adipocytes were assessed following the guidelines provided with respective detection kits.

RNA Extraction and qRT-PCR

Total RNA was extracted from 3T3L1 cells using Trizol (15596018, Invitrogen, Carlsbad, CA, USA), followed by reverse transcription into cDNA utilizing the Takara PrimeScript RT Reagent Kit (RR037A, Takara, Kusatsu, Japan). Utilizing iScript Reverse Transcription (1708891, BioRad, Redmond, WA, USA), 2 µg of total RNA underwent reverse transcription to generate cDNA. Furthermore, qRT-PCR was conducted employing SsoAdvanced Universal SYBR Green Supermix (1725270, BioRad, Redmond, WA, USA). The amplification conditions were set as follows: initial denaturation at 95 °C for 10 minutes, followed by 40 cycles of denaturation at 95 °C for 30 seconds, annealing at 55–57 °C for 30 seconds, and elongation at 72 °C for 30 seconds [17]. Relative expression levels were assessed using the $2^{-\Delta\Delta Ct}$ method. The primers used in qPCR are shown in **Supplementary Table 2**.

Western Blotting

After cracking cells or tissues with ice-cold lysate (P0013B, Beyotime, Shanghai, China), adipose tissue and treated cell protein were extracted and quantified following the manufacturer's instructions (A045-4-2, BioRad, Redmond, WA, USA). After this, 10 µg of total proteins were resolved using 4–12% Bis-Tris polyacrylamide gel electrophoresis and were transferred onto a polyvinylidene difluoride (PVDF) membrane (03010040001, Millipore, Bellevue, WA, USA). The PVDF membrane was blocked with 5% skim milk for 1 hour. The membrane underwent overnight incubation with monoclonal rabbit antibodies against perilipin A (ab172907, 1:1000, Abcam, Cambridge, UK), ATGL (ab207799, 1:10000, Abcam, Cambridge, UK), and HSL (ab109400, 1:50000, Abcam, Cambridge, UK) at 4 °C. The next day, the mem-

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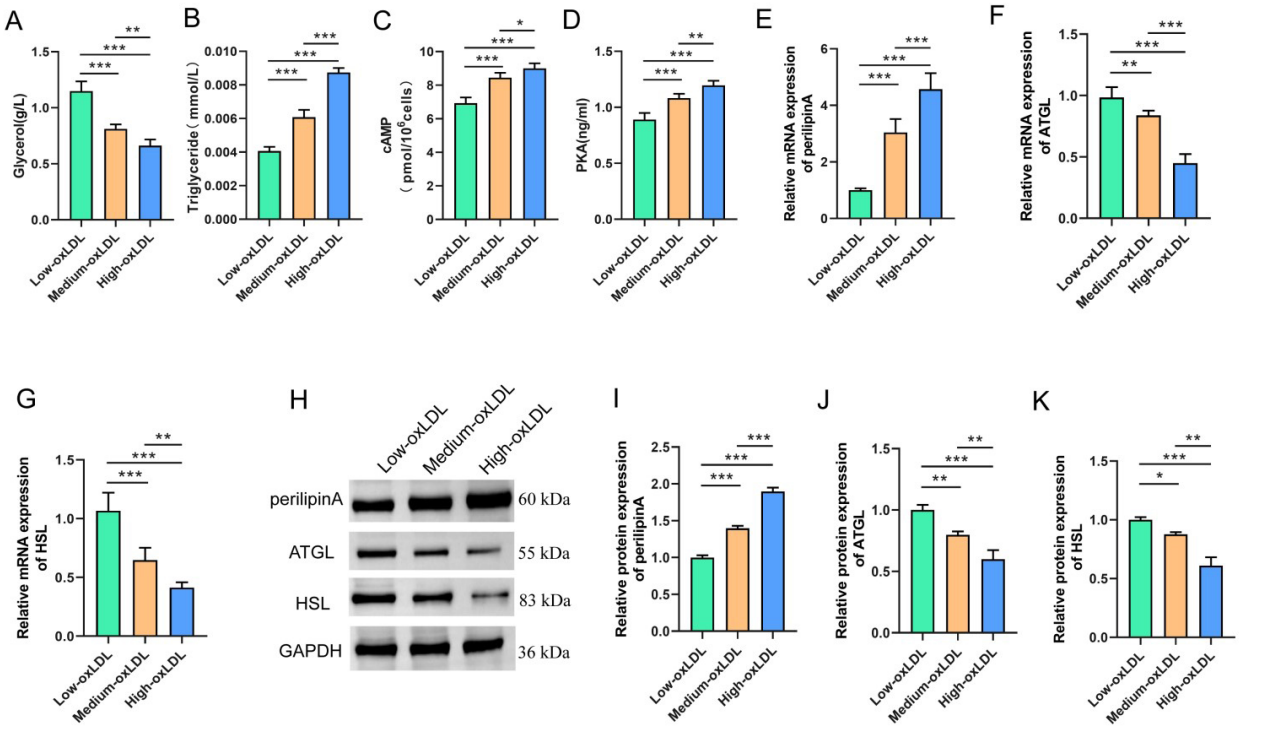

Fig. 2. Expression changes in catabolism-related indexes of triglycerides in 3T3L1 cells treated with various concentrations of oxidized low-density lipoprotein (ox-LDL). The contents of glycerol (A), triglyceride (B), Camp (C), and PKA (D) in 3T3L1 cells treated with ox-LDL at different concentrations were assessed using corresponding biochemical kits. Western blotting and qRT-PCR were employed to observe the changes in the protein and mRNA expression of perilipin A, *ATGL*, and *HSL* in 3T3L1 cells treated with diverse concentrations of ox-LDL (E–K), respectively (n = 3). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

brane was washed three times with Tris-buffered saline, and the blots underwent 1.5-hour incubation with horseradish peroxidase-conjugated secondary anti-rabbit or anti-mouse IgG antibodies (515-475-003, Jackson Immuno Research, Philadelphia, PA, USA) at ambient temperature. The protein bands were visualized using an enhanced chemiluminescence system (1705061, BioRad, Redmond, WA, USA). Finally, the protein bands were quantified utilizing Image J densitometry software (1.49, NIH, Bethesda, MA, USA), and the findings were expressed as relative fold change of treatment compared to the internal reference *GAPDH* (1:5000), following normalization against a housekeeping protein.

Immunofluorescence

The cells were exposed to varying concentrations of ox-LDL, LPS, and glucose for 2 days. After this, the cells were fixed in 4% paraformaldehyde for 30 minutes, followed by three washes with PBS for 5 minutes each. In the next step, 50 µL of membrane permeabilization solution was added and incubated at room temperature for 10 minutes, and underwent three additional washes with PBS for 5 min each. The cells were then incubated overnight with the primary antibody, perilipin A (LM3789R, LMAI Bio, Shanghai, China), and the secondary antibody, IgG (SAB3700885, Sigma-Aldrich, St. Louis, MO, USA). After this, the nucleus was stained with 4′,6-Diamidino-2′-phenylindole (DAPI, D1306, Invitrogen, Carlsbad, CA, USA), and the samples were sealed with 1% gelatin before being observed using a laser scanning confocal microscope (LSM 880 NLO with Airyscan, Carl Zeiss AG, Jena, Germany). Furthermore, the morphology of lipid droplets in 3T3L1 adipocytes following infection with Bodipy493/503 (CAS#:121207-31-6, Sigma-Aldrich, St. Louis, MO, USA) for 2 days after various treatments was analyzed utilizing a confocal microscope. Subsequently, the lipid droplets underwent 3D scanning and enumeration.

Statistical Analysis

Statistical analyses were conducted using SPSS 22.0 (IBM, Armonk, NY, USA). Each experiment was performed in triplicates. The data were expressed as the mean ± standard error of the mean (SEM). Comparison between the two groups was achieved using a *t*-test. Moreover, multiple group comparisons were conducted utilizing One-way ANOVA, followed by Dunnett's test. Statistical significance was considered at a *p*-value < 0.05. Additionally, the figures were plotted through the GraphPad Prism (version 5.0, GraphPad Software Inc, San Diego, CA, USA).

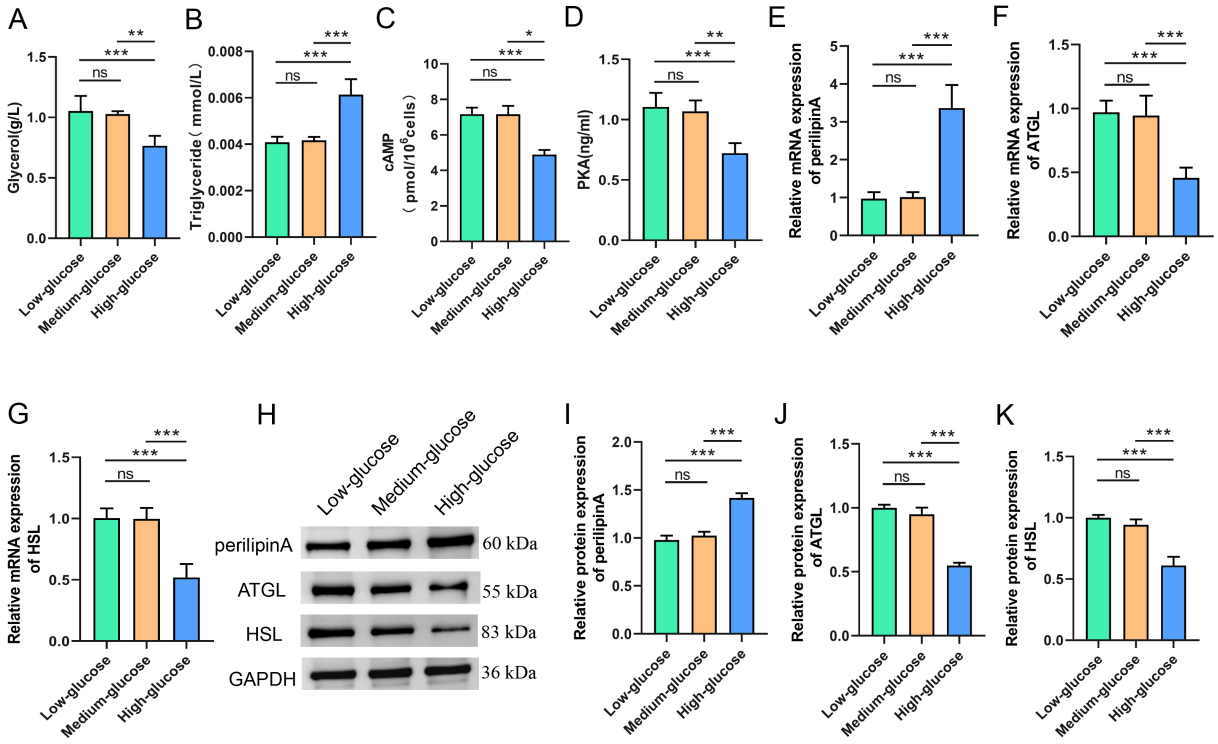

Fig. 3. Expression levels of catabolism-related indexes of triglycerides in 3T3L1 cells treated with varying concentrations of glucose. The contents of glycerol (A), triglyceride (B), cAMP (C), and PKA (D) in 3T3L1 cells exposed to various concentrations of glucose were determined utilizing corresponding biochemical kits. Western blotting and qRT-PCR were used to assess the protein and mRNA expression levels of perilipin A, ATGL, and HSL in 3T3L1 cells treated with different concentrations of glucose (E–K), respectively (n = 3). ns, no significance, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Results

Expression Levels of Glycerol, Triglyceride, Perilipin A, cAMP, PKA, ATGL, and HSL in the CHD Group

The expression levels of glycerol ($p < 0.01$), cAMP ($p < 0.001$), PKA ($p < 0.001$), ATGL ($p < 0.05$), and HSL ($p < 0.05$) in EAT were significantly decreased in the CHD group compared to the non-CHD group (Fig. 1A–E). Conversely, the triglyceride content and perilipin A expression were significantly increased ($p < 0.05$, Fig. 1F,G). These findings indicate abnormal metabolism of EAT in the CHD group.

Expression Levels of Glycerol, Triglyceride, Perilipin A, cAMP, PKA, ATGL, and HSL in 3T3L1 Cells Treated with Different Concentrations of ox-LDL

The findings of the biochemical test demonstrated that compared to the low-ox-LDL group, the glycerol content in both the medium-ox-LDL and high-ox-LDL groups of cells was significantly reduced, accompanied by a substantial increase in triglyceride, cAMP, and PKA content ($p < 0.001$). Moreover, relative to the medium-ox-LDL group, the triglyceride, cAMP, and PKA levels were elevated in the high-ox-LDL group, accompanied by a substantial reduction in glycerol level (Fig. 2A–D). The findings from qRT-PCR and western blotting revealed that compared to the low-ox-LDL group, the medium-ox-LDL and high-ox-LDL groups of cells exhibited significantly increased levels of perilipin A mRNA and protein expression ($p < 0.001$), while the expression levels of *HSL* and *ATGL* mRNA and protein were substantially reduced ($p < 0.001$). Furthermore, the expression level of perilipin A was significantly higher in the high-ox-LDL group of cells compared to the medium-ox-LDL group; the expression levels of *ATGL* and *HSL* were substantially reduced (Fig. 2E–K). These findings indicate that high concentrations of ox-LDL can inhibit the lipolysis process in 3T3L1 adipocytes.

Expression Levels of Glycerol, Triglyceride, Perilipin A, cAMP, PKA, ATGL, and HSL in 3T3L1 Cells Treated with Various Glucose Concentrations

The findings from the biochemical analysis revealed that compared to the low-glucose group, there was a significant reduction in the levels of glycerol, cAMP, and PKA in high-glucose-treated 3T3L1 cells ($p < 0.05$). In contrast, the triglyceride level showed a substantial increase ($p < 0.05$). Additionally, triglyceride level in the high-glucose group was significantly increased relative to the middle-glucose group, while the levels of glycerol, cAMP, and PKA were significantly reduced (Fig. 3A–D).

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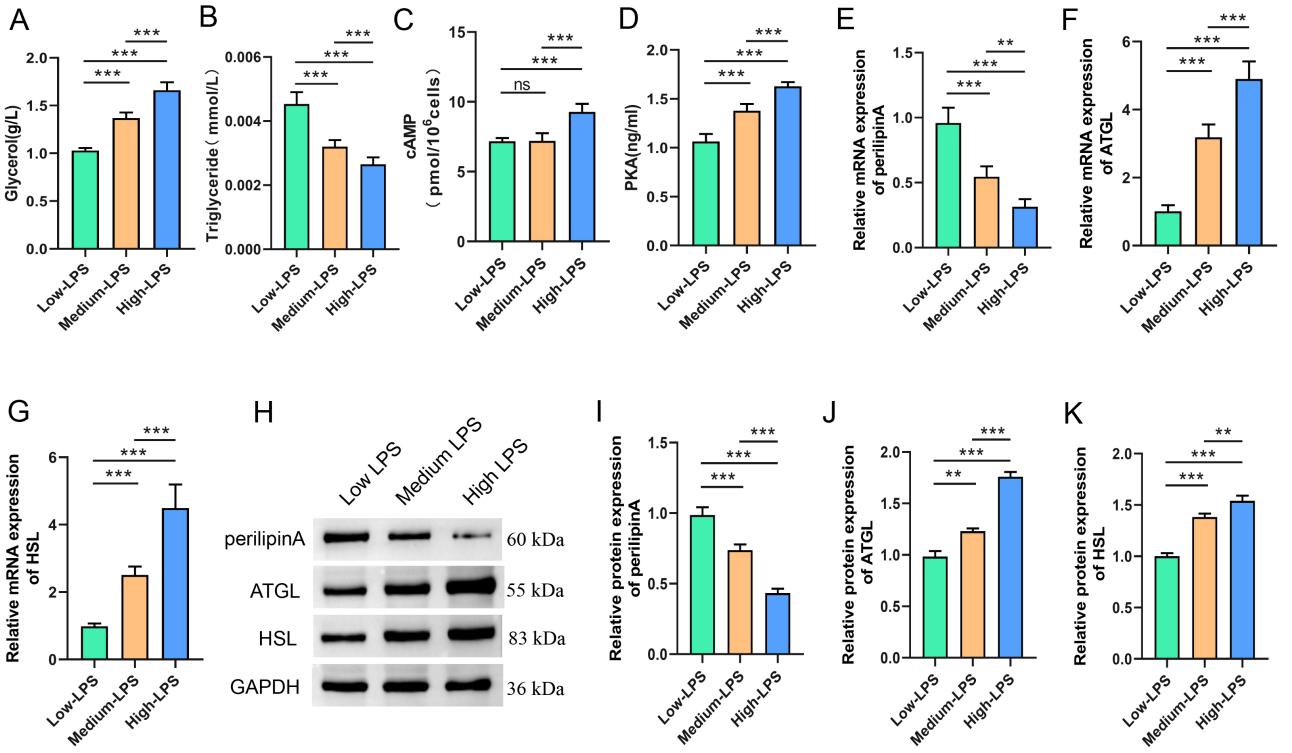

Fig. 4. Expression levels of catabolism-related indexes of triglycerides in 3T3L1 cells treated with various concentrations of lipopolysaccharide (LPS). The contents of glycerol (A), triglyceride (B), cAMP (C), and PKA (D) in 3T3L1 cells induced with varying concentrations of LPS were assessed using biochemical kits. Western blotting and qRT-PCR analyses were utilized to observe the expression levels of protein and mRNA of perilipin A, ATGL, and HSL in 3T3L1 cells after treatment with LPS at varying concentrations (E–K), respectively (n = 3). ns, no significance, $^{**}p < 0.01$, $^{***}p < 0.001$.

The qRT-PCR and western blotting results demonstrated a substantial increase in perilipin A expression levels in 3T3L1 cells exposed to high-glucose conditions relative to those in the low-glucose group ($p < 0.001$). Conversely, the expression levels of ATGL and HSL were substantially diminished in the high-glucose group compared to the low-glucose group ($p < 0.001$). A significant elevation in perilipin A expression levels was observed in 3T3L1 cells treated with the high-glucose group compared to those in the medium-glucose group ($p < 0.001$). Furthermore, the high-glucose group exhibited significantly lower expression levels of ATGL and HSL compared to the medium-glucose group ($p < 0.001$, Fig. 3E–K). These observations suggest that high glucose concentration inhibits the lipolysis process in 3T3L1 adipocytes.

Expression Levels of Glycerol, Triglyceride, Perilipin A, cAMP, PKA, ATGL, and HSL in 3T3L1 Cells Exposed to Different Concentrations of LPS

Biochemical analysis demonstrated that compared to the low-LPS group, the levels of glycerol and PKA were significantly increased in 3T3L1 cells induced by medium-LPS and high-LPS ($p < 0.001$), while the triglyceride content notably decreased ($p < 0.001$). Furthermore, in comparison with the medium-LPS group, the triglyceride levels decreased significantly in the high-glucose group, while the glycerol, cAMP, and PKA levels increased significantly ($p < 0.001$, Fig. 4A–D). The qRT-PCR and western blotting analyses indicated that compared to the low-LPS group, the expression levels of perilipin A were substantially alleviated in 3T3L1 cells mediated with the medium-LPS and high-LPS ($p < 0.01$), while the expression levels of ATGL and HSL were increased ($p < 0.01$). Additionally, relative to the medium-LPS group, the perilipin A expression level in 3T3L1 cells within the high-glucose group decreased considerably, whereas the ATGL and HSL expression levels increased ($p < 0.01$, Fig. 4E–K). Consequently, a high concentration of LPS promoted the lipolysis of 3T3L1 adipocytes.

The Expression Levels of Perilipin A in 3T3L1 Adipocytes Following Various Treatments

As illustrated in Fig. 5A, the immunofluorescence analysis revealed a significant upregulation in perilipin A levels in 3T3L1 cells treated with both the medium-ox-LDL and high-ox-LDL relative to the low-ox-LDL group ($p < 0.05$). Moreover, there was a significant increase in perilipin A levels in the high-ox-LDL group compared to

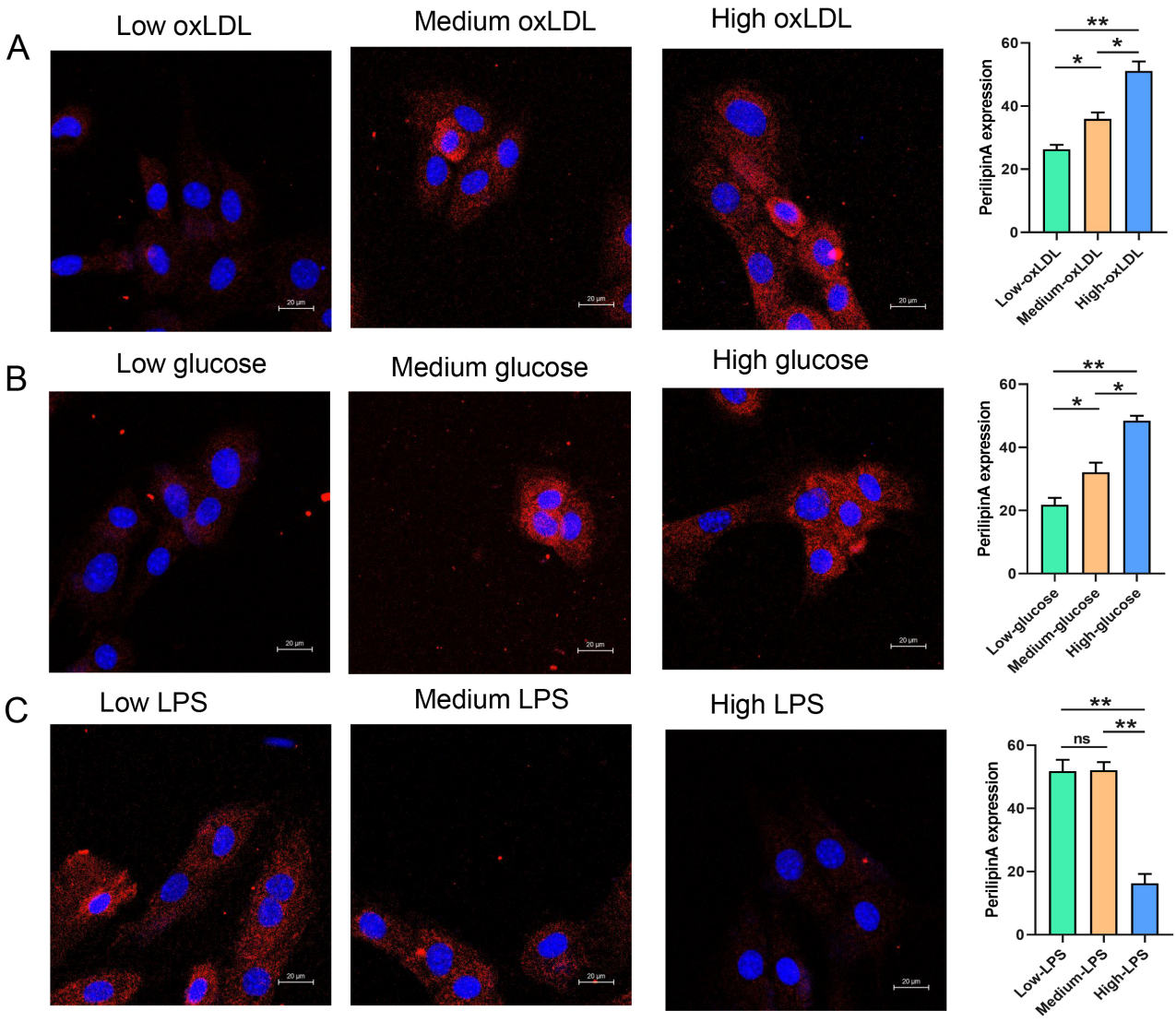

Fig. 5. The expression levels of perilipin A in 3T3L1 adipocytes following various treatments. (A–C) Immunofluorescence was used to assess the expression levels of perilipin A in 3T3L1 cells induced by different concentrations of ox-LDL (A), glucose (B), and LPS (C). Scale bar = 20 μm (n = 3). ns, no significance, *$p < 0.05$, **$p < 0.01$.

the medium-ox-LDL group ($p < 0.05$). Furthermore, relative to the low-glucose group, the intermediate-glucose and high-glucose groups exhibited a substantial elevation in the perilipin A levels ($p < 0.05$). Additionally, a significant increase in perilipin A expression levels within 3T3L1 cells was found in the high-glucose group relative to the intermediate-glucose group ($p < 0.05$, Fig. 5B). Furthermore, a considerable reduction was observed in the levels of perilipin A within the high-LPS group compared to the low-LPS group ($p < 0.01$). Additionally, there was a considerable alleviation in perilipin A levels within the high-glucose group compared to those within the intermediate-glucose group ($p < 0.01$, Fig. 5C).

Changes in the Lipid Droplets within 3T3L1 Adipocytes under Different Treatment Conditions

The immunofluorescence analysis revealed that compared to the low-ox-LDL group, the total number of lipid droplets was significantly increased in the high-ox-LDL group of cells, with a substantial reduction in small lipid droplets and a substantial increase in large lipid droplets ($p < 0.05$). Moreover, compared to the intermediate-ox-LDL group, the total number of lipid droplets increased in 3T3L1 cells after high ox-LDL treatment, along with the alleviation in small lipid droplets (Fig. 6A).

Compared to the low glucose group, the high glucose group exhibited a significant increase in both the total number of lipid droplets and the number of large lipid droplets, along with a significant decrease in the small lipid

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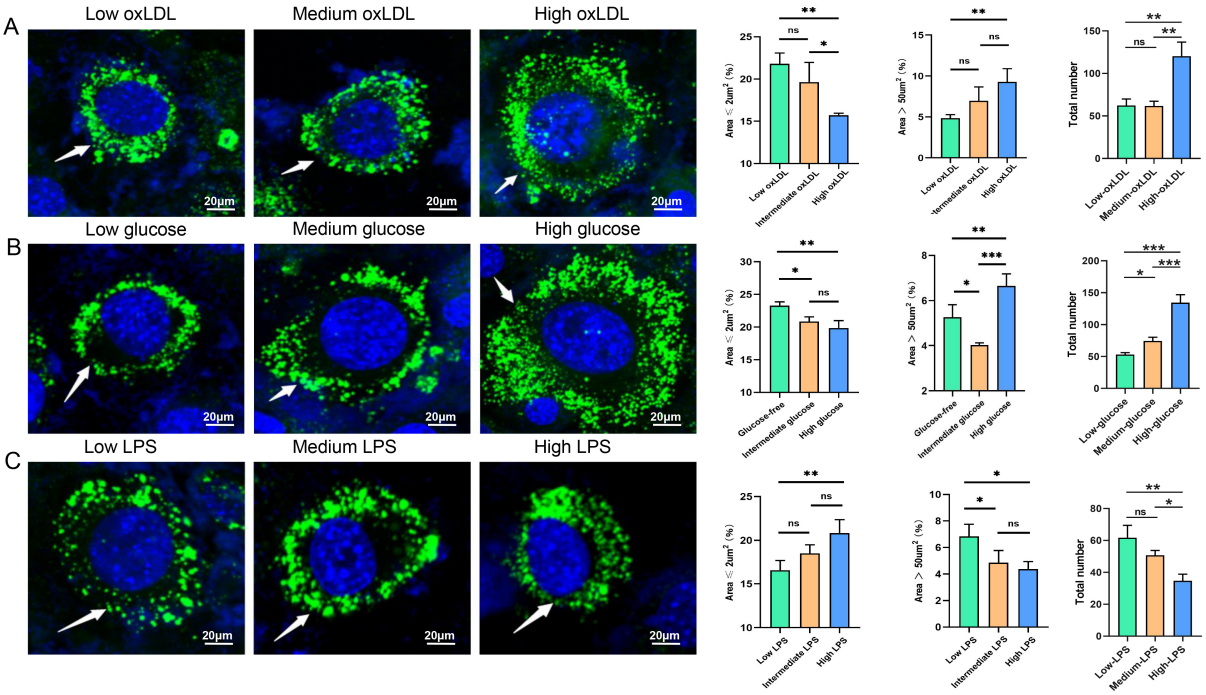

Fig. 6. Changes of lipid droplets within 3T3L1 adipocytes after various treatments (×600). (A–C) Immunofluorescence analysis was used to determine the changes of lipid droplets within 3T3L1 cells following treatments with ox-LDL (A), glucose (B), and LPS (C) at different concentrations. Arrows point to lipid droplets formed by 3T3L1 adipocytes. Scale bar = 20 μm (n = 3). ns, no significance, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

droplets ($p < 0.01$). Compared to the low glucose group, the intermediate glucose group showed a significant increase in the total number of lipid droplets, with a notable decrease in both small and large lipid droplets ($p < 0.05$). Furthermore, compared to the intermediate glucose group, high glucose treatment increased the total number of lipid droplets and large lipid droplets within 3T3L1 cells ($p < 0.001$, Fig. 6B). When compared to the low-LPS group, the high-LPS group indicated a significant decrease in total lipid droplets and large lipid droplets number within 3T3L1 cells, while the number of small lipid droplets was considerably up-regulated ($p < 0.05$). Conversely, compared to the intermediate LPS group, the total number of lipid droplets substantially decreased within 3T3L1 cells treated with high-LPS ($p < 0.05$, Fig. 6C).

Discussion

In adipose tissue, about 80% of its composition is fat, with over 90% of lipids stored as triglycerides [18]. Classical lipolysis primarily involves the hydrolysis of triglycerides into glycerol and fatty acids, mediated by the action of MGL, HSL, and ATGL [7]. Among them, ATGL is highly expressed in adipose tissue, and its expression significantly increases during adipocyte differentiation. Two phosphorylation sites, Ser 404 and Ser 428, have been identified within the C-terminal region of ATGL [19]. Moreover, the enzyme's activity and interaction with CGI-58 depend on the C-terminal domain. High G0S2 expression levels are observed in both adipose tissue and differentiated adipocytes. Specifically, G0S2 interacts with ATGL, leading to the inhibition of its TAG hydrolase activity [20]. Further evidence has validated the regulatory role of G0S2 in human lipolysis. G0S2 exerts an impact on both the activity and intracellular localization of ATGL through the immobilization of lipase to lipid droplets [21]. Moreover, G0S2 has been found to modulate human lipolysis and affect ATGL activity and intracellular localization by immobilizing the lipase to lipid droplets [22].

Under alkaline conditions, nonphosphorylated perilipids interact with CGI 58 ('α/β hydrolase folding') and are located on the surface of lipid droplets, serving to protect triglycerides from hydrolysis [23]. Upon external stimulation, the body activates PKA through the cAMP-PKA signaling pathway, resulting in the phosphorylation of perilipoproteins. Consequently, CGI 58 will no longer combine with phosphorylated perilipoproteins. CGI 58 is promptly transferred from the surface of lipid droplets to the cytoplasm, where it immediately forms a complex with ATGL. This interaction effectively increases the enzymatic hydrolysis activity of ATGL, initiating the first step of adipocyte decomposition [24]. PKA phosphorylates the Ser 563, Ser 659, and Ser 660 sites on the HSL enzyme, improving its hydrolysis activity, thereby further promoting lipolysis. The hydrolysis of adipocytes occurs through

two pathways: the cAMP-PKA pathway and the perilipin A-ATGL-HSL pathway. Through the interaction of signal molecules and enzymes within these two pathways, the hydrolysis of adipocytes is accomplished collaboratively [25].

In this study, the glycerol expression in EAT was lower in the CHD group than in the non-CHD group. Conversely, the expression of triglyceride was higher, indicating a potential inhibition of EAT hydrolysis in CHD, resulting in fat accumulation. Bhatt *et al*. [26] reported that elevated triglyceride levels are correlated with increased mortality from cardiovascular diseases, possibly due to the inhibition of fat hydrolysis and subsequent fat accumulation, aggravating cardiovascular diseases. Assessing the regulation of the expression of enzymes in adipocyte hydrolysis pathways, particularly cAMP-PKA and perilipin A-ATGL-HSL pathways, demonstrated that the contents of ATGL, HSL, PKA, and cAMP in epicardial adipose tissue within the CHD group were significantly reduced, accompanied by a significant elevation in the level of perilipin A.

Adipocytokines secreted by adipocytes can directly affect the functions of vascular smooth muscle cells and vascular endothelial cells, thereby participating in different inflammatory processes, including arteriosclerosis and thrombosis [27]. Conversely, lipids, sugars, inflammatory factors, or other substances in the blood can also directly affect the metabolism of fat cells [28]. Among them, the presence of abnormal oxidized low-density lipoprotein within the intima of blood vessels can induce a cascade of reactions, including the production of foam cells, disruption of vascular endothelial function, and initiation of inflammatory responses [29].

It has been reported that a significant number of hospitalized coronary artery disease patients possess abnormal blood sugar levels, resulting in a substantially increased proportion of abnormal glucose metabolism among individuals affected with coronary artery disease [30]. Concurrently, coronary artery disease can affect the progress of diabetes in affected individuals [31]. Furthermore, a recent study indicates that lipopolysaccharides can activate inflammatory pathways and induce the release of various inflammatory factors, directly participating in the formation of atherosclerotic plaque and, leading to the dysfunction of multiple cell types [32]. As commonly recognized, obesity, ox-LDL, lipopolysaccharide, and abnormal glucose metabolism are all regarded as risk factors for coronary atherosclerotic heart disease. Among them, obesity is an independent contributor to the occurrence and development of CHD. However, the precise mechanisms by which ox-LDL, LPS, and glucose affect the pathway of triglyceride catabolism in adipocytes remain unclear, particularly whether they concurrently act on the same pathway of lipocyte hydrolysis. By comparing protein and large and small lipid droplets involved in the lipolysis pathway, it was found that high concentrations of ox-LDL and glucose inhibited the hydrolysis of adipocytes.

Conversely, as LPS concentration elevated, glycerol content increased gradually, small lipid droplets increased, large lipid droplets decreased, and the lipolysis of adipocytes enhanced. Interestingly, there was no obvious regulatory effect on protein within the lipolysis pathway. These findings show that high concentrations of ox-LDL and glucose inhibit the hydrolysis of 3T3L1 adipocytes, potentially inducing CHD, aligning with the previous findings [33,34]. Additionally, this study demonstrated that high concentrations of ox-LDL and glucose inhibited the hydrolysis of 3T3L1 adipocytes, with both compounds exerting their impacts through regulation of the cAMP-PKA and perilipin A-ATGL-HSL pathways. However, high concentrations of LPS were observed to promote the hydrolysis of adipocytes through mechanisms not involving the cAMP-PKA and perilipin A-ATGL-HSL pathways. Hu *et al*. [35] have reported that ox-LDL-induced atherosclerosis can be promoted by regulating the cAMP/PKA axis, indicating the existence of other pathways and substances regulating the hydrolysis of adipocytes. Consequently, further study is needed to investigate the specific substance or pathway through which LPS regulates the hydrolysis of 3T3L1 adipocytes.

Conclusions

In summary, this study reveals that high ox-LDL and glucose can inhibit the lipolysis of 3T3L1 adipocytes. This effect is likely medicated by regulating the cAMP-PKA and adiponectin A-ATGL-HSL pathways. Conversely, high level of LPS can promote the hydrolysis of 3T3L1 adipocytes. However, the crucial limitations of this study lie in the lack of elucidation into the specific substance or pathway through which LPS regulates the hydrolysis of 3T3L1 adipocytes, necessitating further study.

Availability of Data and Materials

The datasets generated during and analysed during the current study are available from the corresponding authors on reasonable request.

Author Contributions

YHY, HMY and ZLZ designed the research study. QW, HMY and ZLZ performed the research. QW, HMY and ZLZ collected and analyzed the data. YHY, HMY and ZLZ have been involved in drafting the manuscript. All authors have been involved in revising it critically for important intellectual content. All authors gave final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

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Ethics Approval and Consent to Participate

Approval for this study was obtained from the Medical Ethics Committee of Union Hospital Tongji Medical College, Huazhong University of Science and Technology (2018-S007). The study protocol adhered to the guidelines of the Declaration of Helsinki. All study participants provided informed written consent.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.23812/j.biol.regul.homeost.agents.20243805.342.

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