

Chlamydia pneumoniae disrupts lipid metabolism in human umbilical vein endothelial cells

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Abstract. Atherosclerosis is well established as a chronic inflammatory disorder, and *Chlamydia pneumoniae* is considered to be a risk factor for atherosclerotic development. Endothelial dysfunction, caused by oxidized low-density lipoprotein (ox-LDL) is an early atherosclerotic marker. However, the effect of *C. pneumoniae* on lipid metabolism in vascular endothelial cells is yet to be elucidated. The aim of the present study was to investigate the effects of *C. pneumoniae* on lipid metabolism in human umbilical vein endothelial cells (HUVECs). In the present study, LDL oxidation was found to be significantly induced in the supernatant, but not the cell lysates, of *C. pneumoniae*-infected HUVECs. Furthermore, *C. pneumoniae* infection was observed to increase the levels of total cholesterol and cholesteryl esters in LDL-treated HUVECs. In addition, *C. pneumoniae* was found to upregulate the expression of scavenger receptor A, cluster of differentiation 36 and acyl-coenzyme A: cholesterol acyltransferase 1 mRNA and protein. *C. pneumoniae* was also observed to downregulate the mRNA and protein expression of ATP binding cassette transporter (ABC) A1 and ABCG1 in LDL-treated HUVECs. These results show that *C. pneumoniae* disrupts lipid metabolism in HUVECs.

Introduction

Over recent decades, increasing evidence has suggested that infection may be an important risk factor for the development of atherosclerosis and coronary artery disease (1,2). Epidemiological and clinical studies have demonstrated that infection with *Chlamydia pneumoniae*, an intracellular

organism, is an independent risk factor for cardiovascular diseases (3-5). Endothelial cells, vascular smooth muscle cells and monocytes/macrophages are involved in the atherogenic process, and infected peripheral blood monocytes can transmit the *C. pneumoniae* infection to smooth muscle and endothelial cells (6).

The endothelial layer provides a nonthrombogenic and nonadherent surface for circulating leukocytes, and is required for the maintenance of normal vessel function (7). Furthermore, vascular endothelial cells regulate complex processes, including hemostasis, fibrinolysis, inflammation, vessel tone, blood pressure, lipoprotein metabolism and angiogenesis (8). The accumulation of oxidized low-density lipoprotein (ox-LDL) in the arterial wall can activate the immune system and initiate a pro-inflammatory cascade of events, including the endogenous production of reactive oxygen species (ROS), endothelial dysfunction, the recruitment and trans-endothelial migration of monocytes with consequent transformation into foam cells and the proliferation of vascular smooth muscle cells. These events ultimately lead to atherosclerosis (9-11). Studies have shown that *C. pneumoniae* infection stimulates LDL oxidation and uptake and foam cell formation in macrophages (12,13). Furthermore, a previous study by our group indicated that *C. pneumoniae* induced macrophage-derived foam cell formation through upregulation of the expression of scavenger receptor (SR) A1, cluster of differentiation (CD) 36 and acyl-coenzyme A: cholesterol acyltransferase 1 (ACAT1), and through downregulation of the expression of ATP binding cassette transporter (ABC) A1 and ABCG1 (14). These genes regulate intracellular cholesterol homeostasis in vascular endothelial cells, including cholesterol synthesis, influx and efflux (15-17). However, few studies have investigated the effect of *C. pneumoniae* infection on endothelial lipid metabolism.

Materials and methods

Ethics statement. The present study involving fresh plasma was approved by normolipidemic volunteers and the Wuhan Blood Centre (Wuhan, China; authorization: 2010-8) and conformed to the guidelines outlined in the Declaration of Helsinki.

Reagents and antibodies. HUVECs and epithelial HEP-2 cells were obtained from Tongji Medical College (Wuhan,

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China). *C. pneumoniae* strain AR-39 was purchased from the American Type Culture Collection (Rockville, MA, USA). Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were purchased from Hyclone (Thermo Fisher Scientific, Inc., Waltham, MA, USA). A cell lipid peroxide assay kit was purchased from Genmed Scientifics, Inc. (Shanghai, China). A quantitative polymerase chain reaction (qPCR) kit was purchased from Takara Bio Inc. (Otsu, Shiga, Japan). Fluorescein isothiocyanate (FITC)-conjugated specific anti-chlamydial monoclonal antibodies were obtained from Dako Ltd. (Copenhagen, Denmark), and mouse polyclonal anti-SR-A1 antibodies were obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Rat monoclonal anti-CD36 antibodies were obtained from R&D Systems, Inc. (Minneapolis, MN, USA). Rabbit polyclonal anti-ACAT1 antibodies were obtained from Cayman Chemical Co. (Ann Arbor, MI, USA). Rabbit monoclonal anti-ABCG1 antibodies were obtained from Epitomics (Burlingame, CA, USA). Mouse monoclonal anti-ABCA1 antibodies were obtained from Abcam PLC (Cambridge, UK). Mouse polyclonal anti- β -actin antibodies and horseradish peroxidase (HRP)-conjugated secondary antibodies were obtained from AntGene Biotech Co., Ltd. (Wuhan, China).

Propagation of *C. pneumoniae*. *C. pneumoniae* strain AR-39 was propagated in HEp-2 cells in the presence of 2 μ g/ml cycloheximide using centrifugation-driven infection as described previously (18). Infected HEp-2 cells were harvested subsequent to incubation for 72 h at 37°C and 5% CO₂, and disrupted by freezing, thawing and ultrasonication. Following centrifugation at 500 x g at 4°C for 30 min to remove the cell debris, the supernatant containing elementary bodies was collected and suspended in sucrose-phosphate-glutamic acid buffer and stored at -70°C in aliquots until use. Chlamydial inclusion forming units (IFU) were assessed by counting the chlamydial inclusions formed in the HEp-2 cells using FITC-conjugated anti-chlamydial antibodies.

Infection and culture of HUVECs. HUVECs were seeded onto six-well plates at a density of 1x10⁶ cells per well in DMEM containing 10% FBS at 37°C in 5% CO₂. In the presence of 50 mg/l LDL, HUVECs were cultured alone or with *C. pneumoniae* (1x10⁶ IFU) for 48 h. In certain experiments, HUVECs were incubated in six-well plates with 50 mg/l ox-LDL for 48 h.

LDL isolation and ox-LDL preparation. Human LDL (density, 1.006-1.063 g/ml) was isolated from the blood of healthy donors using density-gradient ultracentrifugation as described previously (19). The isolated LDL was dialyzed in phosphate-buffered saline for 24 h and was condensed using polyoxyl 20,000. The concentration of LDL was measured using the Bradford assay, and the LDL underwent sterile filtration (0.22 μ m) prior to storage in the dark at 4°C for no longer than 2 weeks before use. LDL was analyzed using 4-20% SDS-PAGE gradient gel and Sudan black B staining to reveal a single protein. The preparations of ox-LDL from human plasma were performed as described previously (20). Briefly, LDL was diluted to a concentration of 200 mg/dl in medium M199 and incubated with 5 μ mol/l CuSO₄, CuCl₂ or copper acetate

at 37°C for 24 h, as indicated. The reactions were terminated using the addition of 100 μ mol/l EDTA. The oxidation state of LDL was measured using the PeroXOquant Quantitative Peroxide Assay kit (Pierce Biotechnology, Inc., Rockford, IL, USA). Peroxidation products contained in the LDL and ox-LDL were <5 and 200-300 μ mol/l H₂O₂, respectively. Contamination of LDL and the modified LDL preparations by the lipopolysaccharide (LPS) endotoxin was assessed using a Limulus Amebocyte Lysate kit (BioWhittaker®; Lonza, Walkersville, MD, USA). All LDL preparations had an LPS content of <50 pg/mg protein.

Measurement of thiobarbituric acid reactive substances (TBARS). The determination of LDL oxidation was performed as described previously (21). The determination of TBARS involves the measurement of malondialdehyde (MDA) derived from the hydroperoxidation of unsaturated fatty acids containing three or more double bonds (22). In brief, 2.5 ml 10% trichloroacetic acid was added to a tube with 0.5 ml HUVEC supernatant or cell lysates with or without *C. pneumoniae* infection, in order to precipitate the protein. Following centrifugation, 100 μ l supernatant was mixed with 50 μ l 0.67% TBA. Subsequent to vortexing and boiling for 15 min, samples were cooled under running water. Absorbance was measured at 532 nm in a Perkin Elmer Lambda 2 spectrophotometer (Perkin Elmer, Überlingen, Germany) against a blank control with distilled water. The concentration of oxidation products was determined as MDA (nmol/ml) according to a standard curve of MDA reacting with TBA using the following formula: MDA (nmol/ml) = absorbance x 53.4188. Experiments were repeated three times.

Intracellular cholesterol measurement. Cells were harvested and disrupted using ultrasonication. Intracellular TC and free cholesterol (FC) were detected using zymochemistry as described previously (23). TC and FC content was determined using a standard curve (312.5, 625, 1,250 and 2,500 μ mol/l standard cholesterol) and expressed as μ mol/g protein. CE content was calculated by subtracting the content of FC from that of TC. Experiments were repeated three times.

qPCR analysis. Total RNA was isolated using TRIzol® reagent (Takara Bio, Inc.). cDNA was synthesized using a PrimeScript™ RT Reagent kit (Takara Bio, Inc.) according to the manufacturer's instructions, and qPCR was performed using a Taq polymerase (Takara Bio, Inc.). The oligonucleotide primers for SR-A1, CD36, ACAT1, ABCA1, ABCG1 and β -actin were as follows: SR-A1, 5'-GCAGTTCTCATC CCTCTCAT-3' (forward) and 5'-GGTATTCTCTTGAT TTTGCC-3' (reverse); CD36, 5'-TGCCTCTCCTAG TTGAAAAC-3' (forward) and 5'-GCAACAAACGATCAC CACAC-3' (reverse); ACAT1, 5'-TCCCAGGAATCCCAC TGTA-3' (forward) and 5'-ACGAAGAGCACGGGA TAGAA-3' (reverse); ABCA1, 5'-TATGAGGGCCAGATC ACCTC-3' (forward) and 5'-GCTGGCTTGTTTTGCTT TTC-3' (reverse); ABCG1, 5'-CAGGAAGATTAGACTG TGG-3' (forward) and 5'-GAAAGGGGAATGGAGAGAAG-3' (reverse); β -actin, 5'-GTCCACCTTCCAGCAGATGT-3' (forward) and 5'-CACCTTACCGTTCCAGTTT-3' (reverse).

Western blot analysis. Total protein was extracted from the cells and the protein content was measured in triplicate using the Bicinchoninic Acid Protein Assay kit (Pierce Biotechnology, Inc.). Absorption was measured at 562 nm. Protein samples (60 μ g) were separated using 10% SDS-PAGE and transferred to polyvinylidene fluoride membranes (Invitrogen Life Technologies, Grand Island, NY, USA). Membranes were incubated at 4°C overnight with the following antibodies: Mouse polyclonal anti-SR-A1 (dilution, 1:200), rat monoclonal anti-CD36 (dilution, 1:500), rabbit polyclonal anti-ACAT1 (dilution, 1:200), mouse monoclonal anti-ABCA1 (dilution, 1:1,000), rabbit monoclonal anti-ABCG1 (dilution, 1:2,000) and mouse polyclonal anti- β -actin (dilution, 1:1,000). The membranes were then incubated with HRP-conjugated secondary anti-rabbit, -rat and -mouse antibodies, each at a dilution of 1:2,000, at room temperature for 2 h. Proteins were detected using the enhanced chemiluminescence method. For quantification, SR-A1, CD36, ACAT1, ABCA1 and ABCG1 protein levels were normalized to those of β -actin.

Statistical analysis. Quantitative data are expressed as the mean \pm standard deviation. Direct comparisons between two groups were performed using the Student's t-test. Data from more than two groups were compared using analysis of variance with repeated measures, followed by the Student-Newman-Keuls multiple comparison test. A value of $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Increased lipid peroxidation products in the supernatants, but not cell lysates, of *C. pneumoniae*-infected HUVECs. Lipid peroxidation was quantified using the measurement of TBARS. As shown in Fig. 1A, lipid peroxidation products were increased in the supernatant of *C. pneumoniae*-infected HUVECs (1×10^6 IFU). MDA production was observed to increase from 4.61 ± 0.90 nmol/ml in the control HUVEC supernatant to 7.16 ± 0.40 nmol/ml in the infected HUVEC supernatant ($P < 0.05$). In contrast to the supernatant, no significant difference in LDL oxidation was observed in the infected HUVEC lysates (Fig. 1B; $P > 0.05$).

Effect of *C. pneumoniae* on intracellular cholesterol metabolism. Cholesterol is accumulated either as CE in cytoplasmic vesicles or as FC in the membrane (24). To determine whether intracellular cholesterol metabolism was affected by *C. pneumoniae* in HUVECs, the levels of intracellular TC and CE were assessed using lipid mass quantification. LDL-treated HUVECs were used as a negative control, ox-LDL-treated HUVECs were used as a positive control and LDL-treated HUVECs infected with *C. pneumoniae* were used as the infection group. As shown in Table I, *C. pneumoniae* infection was found to increase the levels of TC and CE compared with the negative control ($P < 0.05$). In addition, levels of intracellular TC and CE were increased in the ox-LDL-treated HUVECs compared with those in the negative control group ($P < 0.05$). *C. pneumoniae* infection was not observed to significantly increase TC and CE levels compared with the positive controls ($P > 0.05$).

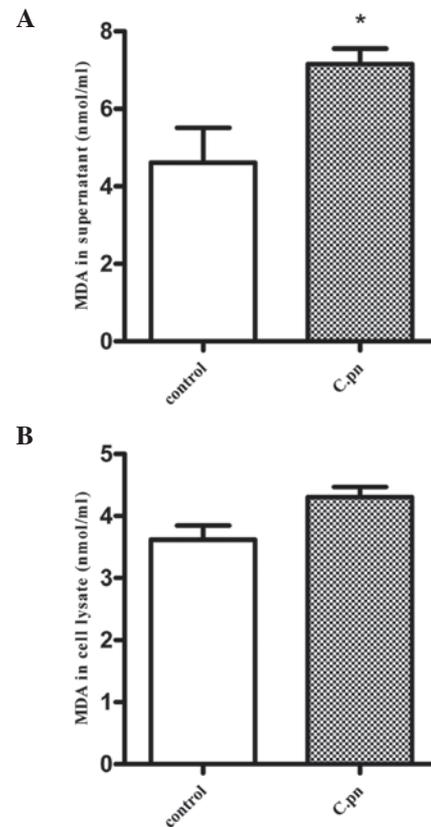


Figure 1. MDA production in the (A) supernatant and (B) cell lysates of control and C.pn-infected HUVECs. In the presence of 50 mg/l LDL, HUVECs were cultured alone or with C.pn (1×10^6 inclusion forming units) for 48 h. The LDL-treated HUVECs were used as controls. The increase in lipid peroxidation products was expressed as an increase in MDA production. Data are presented as the mean \pm standard deviation ($n = 3$). * $P < 0.05$ versus control. HUVECs, human umbilical vein endothelial cells; MDA, malondialdehyde; LDL, low-density lipoprotein; C.pn, *Chlamydia pneumoniae*.

SR-A1 and CD36 expression is upregulated in LDL-treated HUVECs with *C. pneumoniae* infection. The mRNA and protein expression of lipoprotein receptors was assessed using qPCR and western blot analyses, respectively. SR-A1 and CD36 have been demonstrated to participate in lipoprotein uptake (25). As shown in Fig. 2A and B, SR-A1 and CD36 mRNA expression was upregulated by up to 1.8- and 2.0-fold in the *C. pneumoniae*-infected HUVECs compared with that in the negative controls ($P < 0.05$). In addition, ox-LDL was found to upregulate the mRNA expression of SR-A1 and CD36 by up to 2.0- and 1.5-fold, respectively ($P < 0.05$). However, no significant difference was observed in the mRNA expression of SR-A1 and CD36 between the *C. pneumoniae*-infected HUVECs and the positive controls ($P > 0.05$). Furthermore, *C. pneumoniae* infection was found to increase the protein expression of SR-A1 and CD36 by 3.53- and 2.40-fold, respectively, relative to the negative control group ($P < 0.05$). SR-A1 and CD36 protein expression was also increased by up to 4.12- and 2.11-fold, respectively, in the positive control group relative to that in the negative controls ($P < 0.05$) (Fig. 2C and D). No significant difference was observed in the protein expression of SR-A1 and CD36 between the *C. pneumoniae*-infected HUVECs and the positive controls ($P > 0.05$).

Table I. Effect of *Chlamydia pneumoniae* on TC and CE levels in human umbilical vein endothelial cells.

Groups	TC ($\mu\text{mol/g}$)	FC ($\mu\text{mol/g}$)	CE ($\mu\text{mol/g}$)	CE/TC (%)
LDL	35.7 \pm 0.8	24.4 \pm 0.6	11.3 \pm 1.1	31.7
LDL+C.pn	74.5 \pm 3.1 ^a	41.3 \pm 1.7	33.2 \pm 2.1 ^a	44.6
ox-LDL	76.5 \pm 2.6 ^a	39.5 \pm 1.2	36.7 \pm 0.9 ^a	48.2

Data are presented as the mean \pm standard deviation of three independent experiments. ^aP<0.05 versus control (LDL group). TC, total cholesterol; CE, cholesteryl ester; FC, free cholesterol; LDL, low-density lipoprotein; ox-LDL, oxidized LDL; C.pn, *Chlamydia pneumoniae*.

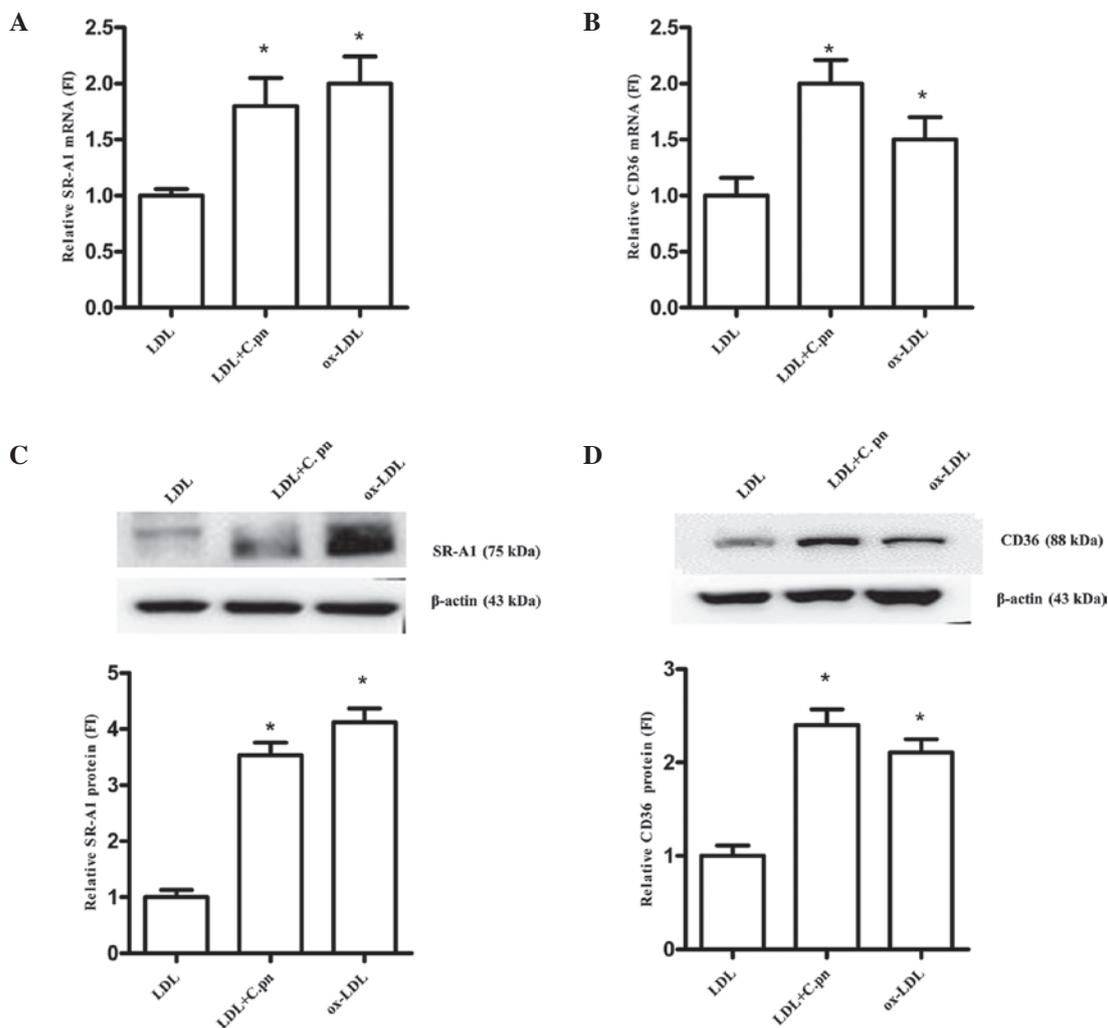


Figure 2. C.pn upregulates SR-A1 and CD36 expression in LDL-treated HUVECs. In the presence of 50 mg/l LDL or ox-LDL, HUVECs were cultured alone or with C.pn (1×10^6 inclusion forming units) for 48 h. (A and B) Quantitative polymerase chain reaction analysis of (A) SR-A1 and (B) CD36 mRNA expression relative to that of β -actin. (C and D) Western blot analysis of (C) SR-A1 and (D) CD36 protein expression relative to that of β -actin. Data are expressed as the FI of the control (control=1 arbitrary unit). Data are presented as the mean \pm standard deviation from three independent experiments. *P<0.05 versus control (LDL group). HUVECs, human umbilical vein endothelial cells; LDL, low-density lipoprotein; ox-LDL, oxidized LDL; C.pn, *Chlamydia pneumoniae*; SR, scavenger receptor; CD, cluster of differentiation; FI, fold induction.

ACAT1 expression is upregulated in LDL-treated HUVECs with *C. pneumoniae* infection. The conversion of cholesterol to CE is catalyzed by the enzyme ACAT. In order to determine the role of ACAT1 in the *C. pneumoniae*-induced perturbation of intracellular cholesterol homeostasis in HUVECs, the expression of ACAT1 was assessed using qPCR and western blot analyses. As shown in Fig. 3A and B, *C. pneumoniae* infection (1×10^6 IFU) was found to significantly increase the

mRNA and protein expression of ACAT1 by up to 1.5- and 1.9-fold, respectively, compared with the LDL-treated HUVECs (P<0.05). In addition, ox-LDL upregulated ACAT1 mRNA expression by up to 1.58-fold and ACAT1 protein expression by up to 2.03-fold relative to the negative control. No significant difference was observed in ACAT1 mRNA and protein expression between the *C. pneumoniae*-infected HUVECs and the positive controls (P>0.05). These data show

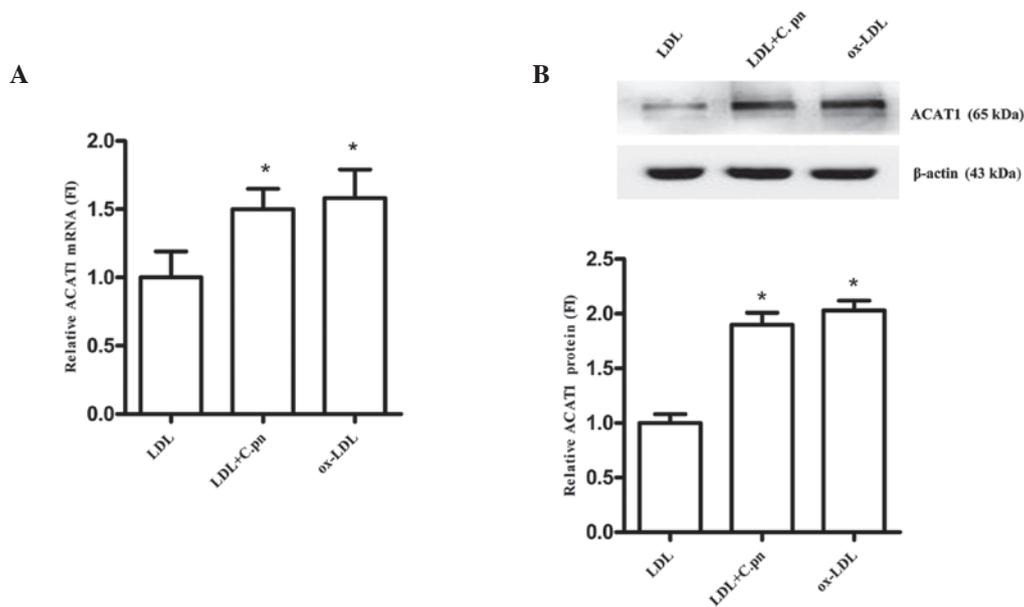


Figure 3. C.pn upregulates ACAT1 expression in LDL-treated HUVECs. In the presence of 50 mg/l LDL or ox-LDL, HUVECs were cultured alone or with C.pn (1×10^6 inclusion forming units) for 48 h. (A) Quantitative polymerase chain reaction analysis of ACAT1 mRNA expression relative to that of β -actin. (B) Western blot analysis of ACAT1 protein expression relative to that of β -actin. Data are expressed as the FI of the control (control=1 arbitrary unit). Data are presented as the mean \pm standard deviation from three independent experiments. * $P < 0.05$ versus control (LDL group). HUVECs, human umbilical vein endothelial cells; LDL, low-density lipoprotein; ox-LDL, oxidized LDL; C.pn, *Chlamydia pneumoniae*; ACAT1, acyl-coenzyme A: cholesterol acyltransferase 1; FI, fold induction.

that ACAT1 may be involved in the *C. pneumoniae*-induced increases in CE levels in HUVECs.

ABCA1 and ABCG1 expression is downregulated in LDL-treated HUVECs with C. pneumoniae infection. ABCA1 and ABCG1 are critical cell surface proteins that mediate endothelial cholesterol efflux (15). In order to investigate the expression and regulation of ABCA1 and ABCG1 in *C. pneumoniae*-infected HUVECs, the mRNA and protein levels of ABCA1 and ABCG1 were detected. As shown in Fig. 4A and B, the mRNA expression of both ABCA1 and ABCG1 was reduced by 40% in the *C. pneumoniae*-infected HUVECs compared with that in the LDL-treated HUVECs ($P < 0.05$). Furthermore, in the ox-LDL-treated HUVECs, the mRNA expression of ABCA1 and ABCG1 was reduced by 50% ($P < 0.05$). In addition, ABCA1 and ABCG1 protein expression was reduced by 62 and 48% in the *C. pneumoniae*-infected HUVECs ($P < 0.05$; Fig. 4C and D). However, no significant difference was observed between the *C. pneumoniae*-infected group and the positive control group ($P > 0.05$).

Discussion

Atherosclerosis is an inflammatory disease in its genesis and progression, and it is initiated by the retention of lipids. The present study revealed that the supernatant of *C. pneumoniae*-infected HUVECs exhibited significant LDL oxidation, and that *C. pneumoniae* increased the intracellular levels of TC and CE through the regulation of target genes affecting lipid metabolism in endothelial cells. The rate of lipid peroxidation was determined using the TBARS method, which revealed that the supernatant, but not the cell lysate, of *C. pneumoniae*-infected HUVECs exhibited significant

LDL oxidation. Furthermore, *C. pneumoniae* infection was found to significantly increase the levels of TC and CE in the LDL-treated HUVECs; this finding was similar to the results for the ox-LDL-treated HUVECs. The present study also demonstrated that *in vitro* infection with *C. pneumoniae* in LDL-treated HUVECs significantly increased the expression of SR-A1, CD36 and ACAT1, which participate in intracellular lipid influx and CE synthesis. In addition, *C. pneumoniae* was observed to decrease the expression of ABCA1/G1, which mediate intracellular lipid efflux. Overall, the present study has indicated that *C. pneumoniae* disrupts lipid metabolism in HUVECs.

The importance of ox-LDL in terms of atherogenesis is well established. ox-LDL inhibits the expression of the constitutive endothelial nitric oxide synthetase enzyme and leads to oxidative stress-induced vascular endothelial cell apoptosis (26,27). The release of ROS induces the expression of adhesion molecules on endothelial cells (28). Several studies have reported that infected monocytes/macrophages are capable of transmitting *C. pneumoniae* infection to endothelial cells and that *C. pneumoniae* infection of macrophages enhances their adhesion to the endothelium (29,30). In addition, ox-LDL has been reported to enhance the expression of vascular adhesion molecules in *C. pneumoniae*-infected endothelial cells (31); the increased expression of vascular adhesion molecules consequently promotes the attraction and adherence of monocytes and T lymphocytes to the endothelium, which is a key process in the progression of arteriosclerosis (32). In the present study, the supernatant of *C. pneumoniae*-infected HUVECs was found to exhibit significant LDL oxidation. Thus, *C. pneumoniae* may induce LDL oxidation and initiate endothelial dysfunction chronically and continuously, eventually leading to atherogenesis.

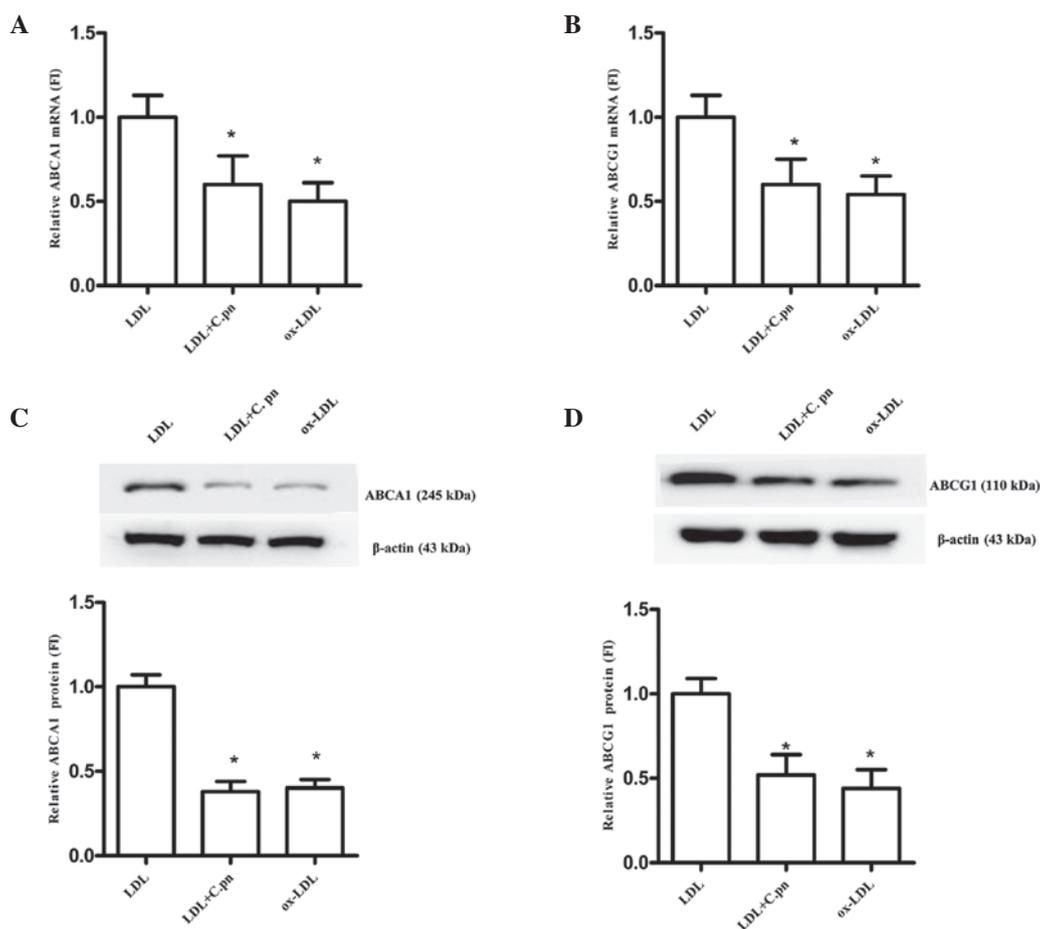


Figure 4. *C.pn* downregulates ABCA1 and ABCG1 expression in LDL-treated HUVECs. In the presence of 50 mg/l LDL or ox-LDL, HUVECs were cultured alone or with *C.pn* (1×10^6 inclusion forming units) for 48 h. (A and B) Quantitative polymerase chain analysis of (A) ABCA1 and (B) ABCG1 mRNA expression relative to that of β -actin. (C and D) Western blot analysis of (C) ABCA1 and (D) ABCG1 protein expression relative to that of β -actin. Data are expressed as the FI of the control (control=1 arbitrary unit). Data are presented as the mean \pm standard deviation from three independent experiments. * $P < 0.05$ versus control (LDL group). HUVECs, human umbilical vein endothelial cells; LDL, low-density lipoprotein; ox-LDL, oxidized LDL; *C.pn*, *Chlamydia pneumoniae*; ABC, ATP binding cassette transporter; FI, fold induction.

Vascular endothelial cells express receptors for oxidized lipoproteins and exhibit biochemical pathways for sterol synthesis and receptor-mediated endocytosis of lipoproteins (33). A variety of SRs that bind to ox-LDL and promote atherogenesis have been identified. SR-A1 and CD36 promote inflammation and cell adhesion, and lectin-type oxidized LDL receptor 1 (LOX-1) mediates and promotes oxidative stress and inflammation (34). A previous study showed that infection of HUVECs with *C. pneumoniae* enhanced the uptake of ox-LDL and the expression of LOX-1, which may directly contribute to atherogenesis (35). In the present study, *C. pneumoniae* infection was found to cause a significant increase in SR-A1 and CD36 expression in HUVECs. These findings suggest that these receptors mediate ox-LDL uptake and lead to the accumulation of intracellular TC.

ABCA1 and ABCG1 are considered to be the most important genes involved in reverse cholesterol transport, through the promotion of high-density lipoprotein (HDL) subfraction 3 and/or apolipoprotein A-I-mediated cellular cholesterol efflux (33). Furthermore, HDL is considered to have an important effect on endothelial health (36). A previous study showed that ox-LDL downregulated ABCA1 in human endothelial cells (37). The present study found that *C. pneumoniae*

infection downregulated the expression of ABCA1 and ABCG1 in HUVECs, which may have mediated a reduction in cholesterol efflux. Therefore, *C. pneumoniae* infection may impair the efflux of excessive intracellular lipids and lead to the accumulation of intracellular TC.

The accumulation of intracellular TC has cytotoxic effects. Oxidized lipids have been shown to induce oxidative stress-induced endothelial cell apoptosis and reduce mitochondrial function (38). Excess cellular cholesterol is stored as CEs. In the majority of cell types, CEs are present only at low levels and predominantly as cytoplasmic lipid droplets. The appearance of CE-enriched lipid droplets in tissues is a consequence of impaired metabolism or overnutrition (39). ACAT1 is a key and exclusive microsomal enzyme that esterifies FC with fatty acids to form CE. ACAT1 is expressed in numerous tissue and cell types, including hepatocytes and Kupffer cells in the liver, adrenal glands, neurons, macrophages, smooth muscle cells and vascular endothelial cells; this expression accounts for >80% of the total ACAT enzyme activity measured *in vitro* (40). The present study revealed that *C. pneumoniae* increased ACAT1 expression and induced the accumulation of intracellular CE and TC. The increase in intracellular TC induced by *C. pneumoniae* in HUVECs may

be a result of increased influx and reduced efflux of cholesterol. The increase in intracellular CE may be mediated by ACAT1 and the accumulation of intracellular CE and TC may lead to endothelial dysfunction in atherogenesis.

In conclusion, the present study has revealed that *C. pneumoniae* significantly induces LDL oxidation and the accumulation of intracellular TC by increasing lipid influx and reducing lipid efflux in HUVECs. In addition, *C. pneumoniae* increases intracellular CE levels. The abnormalities in lipid metabolism induced by *C. pneumoniae* may result in endothelial injury and atherogenesis. These findings may provide an enhanced understanding of the association between infection and atherogenesis.

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