

Insulin promotes macrophage foam cell formation: potential implications in diabetes-related atherosclerosis

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The prevalence of atherosclerotic cardiovascular disease is higher in patients with type 2 diabetes, a disorder characterized by hyperinsulinemia and insulin resistance. The role of hyperinsulinemia as an independent participant in the atherogenic process has been controversial. In the current study, we tested the effect of insulin and the insulin sensitizer, adiponectin, on human macrophage foam cell formation. We found that both insulin and adiponectin increased the expression of the type 2 scavenger receptor CD36 by approximately twofold and decreased the expression of the ATP-binding cassette transporter ABCA1 by >80%. In both cases regulation was post-transcriptional. As a consequence of these changes, we found that oxidized LDL (oxLDL) uptake was increased by 80% and cholesterol efflux to apolipoprotein A1 (apoA1) was decreased by ~25%. This led to two- to threefold more cholesterol accumulation over a 16-h period. As reported previously in studies of murine systems, scavenger receptor-A (SR-A) expression on human macrophages was downregulated by insulin and adiponectin. Insulin and adiponectin did not affect oxLDL-induced secretion of monocyte attractant protein-1 (MCP-1) and interleukin-6 (IL-6). These studies suggest that hyperinsulinemia could promote macrophage foam cell formation and thus may contribute to atherosclerosis in patients with type 2 diabetes.

Laboratory Investigation (2012) 92, 1171–1180; doi:10.1038/labinvest.2012.74; published online 23 April 2012

KEYWORDS: ABCA1; atherosclerosis; CD36; foam cell; insulin; macrophage

Cardiovascular disease is the leading cause of death in many developed countries, and atherosclerosis accounts for most of the major pathology.^{1,2} Patients with type 2 diabetes mellitus, a condition characterized by insulin resistance and compensatory hyperinsulinemia, have a two- to threefold increased risk of atherosclerotic cardiovascular disease.^{3–5} Although there have been many studies that support the causative role of insulin resistance in cardiovascular disease from both epidemiologic and experimental perspectives,^{6–11} there is very little evidence supporting a direct cause and effect relationship between hyperinsulinemia and atherosclerosis. Furthermore, the role of hyperinsulinemia as an independent risk factor has been controversial. Several prospective population studies including the Quebec Cardiovascular study showed an association of high plasma insulin levels with increased risk of coronary heart disease,^{12–17} but other studies, such as that of Welin *et al*¹⁸ failed to show such

an association.¹⁹ As hyperinsulinemia usually occurs in states of insulin resistance it is difficult to determine an independent role for hyperinsulinemia in the pathogenesis of atherosclerosis.

Excessive lipid accumulation by macrophages has a crucial role in the initiation and progression of atherosclerosis. Lipid laden macrophage foam cells accumulate in atheromatous plaque and promote inflammation by secreting cytokines that recruit other immune cells to the arterial intima. Foam cells are generated by uncontrolled uptake of modified LDL, especially oxidized LDL (oxLDL) and/or impaired cholesterol efflux.^{20,21} Lipid homeostasis in macrophages is regulated by scavenger receptors, including CD36 and scavenger receptor-A (SR-A), which mediate uptake and specific ATP-binding cassette (ABC) family transporters that mediate cholesterol efflux to apolipoprotein A1 (apoA1) and high-density lipoprotein (HDL).^{22–25} Thus, alteration in expression of these

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Received 9 August 2011; revised 29 December 2011; accepted 9 February 2012

molecules in macrophages may affect foam cell formation and progression of atherosclerosis.

Adiponectin, also known as Acrp30, is an adipokine exclusively expressed and secreted by adipocytes that functions as an insulin sensitizer. Plasma concentrations of adiponectin are low in type 2 diabetic patients^{26,27} and mice lacking adiponectin have hepatic insulin resistance.²⁸ Administration of adiponectin improves insulin sensitivity in animal models of type 2 diabetes and insulin resistance.^{29,30} The precise molecular mechanism by which adiponectin sensitizes cells to insulin signals has not been elucidated, however, it appears to include cross-talk between adiponectin and insulin receptor (IR) signaling pathways.³¹ Adiponectin was recently suggested to have an anti-atherogenic effect through regulation of SR-A and acyl-coenzyme A:cholesterol acyltransferase-1 (ACAT-1) expression in macrophages.^{32,33}

In the current study, we used human peripheral blood monocyte-derived macrophages to test the effect of insulin and adiponectin on macrophage expression of scavenger receptors and ABC transporter sub-family A member 1 (ABCA1) and on oxLDL uptake, cholesterol efflux and foam cell formation. We found that insulin and adiponectin up-regulated CD36 expression and downregulated ABCA1 expression, resulting in enhanced oxLDL uptake, diminished cholesterol efflux and increased foam cell formation.

MATERIALS AND METHODS

Reagents

LDL prepared from human plasma was oxidatively modified as previously described using a myeloperoxidase, glucose oxidase, nitrite system.³⁴ Bovine insulin was from Sigma and recombinant human adiponectin from R&D systems. Polyclonal antibody against human CD36 was from Cayman Chemical. Monoclonal anti-human CD36 IgG, phycoerythrin-conjugated anti-CD36 IgG and antibodies against SR-A, actin and α -tubulin were from Santa-Cruz Biotechnology. Antibodies against ABCA-1 and EMR1 (F4/80) were purchased from Abcam. C¹⁴-labeled cholesterol was purchased from American Radiolabeled Chemicals. ApoA1 protein was prepared as previously described.³⁵ Quantikine Colorimetric Sandwich ELISA kits for IL-6 and monocyte chemoattractant protein-1 (MCP-1) were from R&D systems.

Cells

Human monocytes were isolated from peripheral blood by Ficoll-Hypaque centrifugation and were cultured in RPMI containing human AB serum (10%) for 7 days to allow for macrophage differentiation. The differentiation of the monocytes into macrophages was confirmed by flow cytometry with anti-EMR1(F4/80) antibody. Human peripheral blood was donated by non-diabetic healthy volunteers. Each sample was screened and the absence of hepatitis B, hepatitis C and HIV infection was confirmed.

Immunoblot Analyses

Human macrophages incubated with different concentrations of insulin (300 pM, 2 nM, 100 nM), adiponectin (2 μ g/ml), oxLDL (50 μ g/ml), LY294002 (10 μ M) or wartmannin (100 nM) for 16 h were lysed with buffer containing 1% triton X-100. Lysates were separated by SDS-PAGE, transferred to PVDF membranes (Millipore) and probed with antibodies against CD36, ABCA-1, SR-A, actin or α -tubulin. Band intensities were quantified by ImageJ (<http://rsbweb.nih.gov/ij/>), Image-Pro Plus software (Media Cybernetics) and Gel-Pro Analyzer (Media Cybernetics).

Flow Cytometry

Human macrophages plated on serum-coated glass coverslips were incubated with insulin, adiponectin or oxLDL for 16 h, then fixed with 4% paraformaldehyde in PBS. Treated macrophages were gently scraped and collected in microtubes and then stained with PE-conjugated anti-CD36 or anti-SR-A IgG before measuring fluorescence intensity by flow cytometry with a Becton-Dickinson FACScan. Data were analyzed by FlowJo software (Tree Star). To assess oxLDL uptake, oxLDL was labeled with the fluorescent probe 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanide perchlorate (diI; Molecular Probes) as previously described.³⁶ Macrophages pre-incubated as above with insulin or adiponectin for 16 h were then exposed to diI-oxLDL (10 μ g/ml) for 20 min and then fixed with 4% paraformaldehyde and analyzed by laser confocal microscopy. Fluorescence uptake was quantified using Image-Pro Plus software (Media Cybernetics).

RT-PCR

Total RNA was isolated by Tri reagent from human macrophages treated with insulin, adiponectin or oxLDL for 16 h and was converted into cDNA by reverse transcriptase (Roche) with oligo-dT primer. cDNAs were then used for PCR with the primers 5'-CAG AGG CTG ACA ACT TCA CAG-3', 5'-AGG GTA CGG AAC CAA ACT CAA-3' for CD36 or the primers 5'-AAC TCT ACA TCT CCC TT CCC G -3', 5'-TGT CCT CAT ACC AGT TGA GAG AC-3' for ABCA-1. PCR for actin was used as a reference with the primers 5'-GTG GGG CGC CCC AGG CAC CA-3', 5'-CTC CTT AAT GTC ACG CAC GAT TTC-3'. PCR amplification was 22 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 2 min for CD36, 28 cycles at the same temperature for ABCA-1 and 17 cycles for actin.

Cholesterol Efflux Assay

Macrophages plated on 24-well dishes were treated with insulin (2 nM, 100 nM) or adiponectin (2 μ g/ml) with or without wartmannin (100 nM) for 16 h. OxLDL (50 μ g/ml) was incubated with C¹⁴-labeled cholesterol (0.2 μ Ci/ml) at 37°C for 30 min and then loaded onto the macrophages. After 6 h, C¹⁴-cholesterol-labeled cells were washed with PBS and incubated with RPMI 1640 medium for 16 h. C¹⁴-cholesterol released from the cells into the medium was measured using scintillation counter. Cellular cholesterol was

extracted by hexane:isopropanol (3:2 v/v), and C^{14} radioactivity in the extract solution was measured by scintillation counter. Efflux percentage was calculated as C^{14} radioactivity in medium/(C^{14} radioactivity in medium + C^{14} radioactivity in cells) \times 100%.

Intracellular Cholesterol Measurement

Human macrophages plated in a 6-well dish were incubated with insulin (2 nM, 100 nM) or adiponectin (2 μ g/ml) with or without LY294002 (10 μ M). The cells were treated with oxLDL for 16 h and lysed by 0.5% triton X-100 containing buffer on ice. The lysates were centrifuged at 17 000 g for 30 min at 4°C and the supernatant was collected for cholesterol measurement. Cholesterol was measured using Cayman cholesterol assay kit (Cayman chemical). Briefly, the cell lysates were mixed with assay buffer containing cholesterol esterase, cholesterol oxidase, HRP and ADHP (10-acetyl-3,7-dihydroxyphenoxazine). Fluorescent product resorufin that was generated by the reaction between ADHP and hydrogen peroxide from cholesterol oxidation could be measured by fluorescence plate reader using excitation wavelengths of 530–580 nm and emission wavelengths of 585–595 nm. We also measured total cholesterol and free cholesterol of macrophages by using gas chromatography coupled with mass spectrometry (GC-MS). Human macrophages were incubated with insulin (2 nM, 100 nM) or adiponectin (2 μ g/ml) with or without wartmannin (200 nM) and then treated with oxLDL (50 μ g/ml) for 16 h. These cells were resuspended with 900 μ l water and 100 μ l of 1 μ g/ml coprosterol in isopropanol and applied to the GC-MS as described previously.³⁷ We recorded the total ion mass spectra of trimethylsilyl derivatives, extracted the GC chromatograms and calculated cholesterol content in each samples. The intracellular cholesterol of each sample was normalized by protein concentration of each sample.

RESULTS

Insulin and Adiponectin Alter Scavenger Receptor Expression in Human Monocyte-Derived Macrophages

Immunoblots for CD36 showed that insulin at 2 and 100 nM and adiponectin at 2 μ g/ml increased CD36 expression in macrophages. The adiponectin concentration used was the one that increased phosphorylation of Akt Ser473 to the same extent as did 100 nM insulin (data not shown). The increase in CD36 was prevented by pre-treatment of the cells with the PI3-kinase inhibitors LY294002 or wartmannin (Figure 1a). The inhibitors themselves had no effect (Figure 1a), unlike what was previously reported for murine macrophages.³⁸ Although the magnitude of effect on CD36 expression induced by insulin or adiponectin varied among cells from different donors, cumulative data from 15 different subjects showed a mean increase of twofold (Figure 1b; $P < 0.05$).

Using flow cytometry we showed that the increase in total CD36 protein levels induced by insulin or adiponectin were

associated with a significant, dose-dependent increase in macrophage cell surface CD36 expression (Figure 1c). Cumulative data from five different subjects showed that 100 nM of insulin and 2 μ g/ml of adiponectin induced mean increases of 1.36-fold and 1.47-fold, respectively (Figure 1d; $P < 0.001$). CD36 mRNA levels measured by RT-PCR did not change after macrophages were exposed to insulin or adiponectin (Figure 1e), suggesting that CD36 regulation was post-transcriptional. OxLDL, known to upregulate CD36,^{39,40} had additive effect in CD36 increase when combined with insulin or adiponectin (Figure 1f).

SR-A, the other major scavenger receptor on macrophages for modified LDL,⁴¹ was also regulated by insulin, but in the opposite direction to CD36. Immunoblots revealed that insulin induced a dramatic, dose-dependent decrease in the expression of SR-A (Figure 2a). Immunofluorescence flow cytometry showed that insulin downregulated SR-A surface expression and that adiponectin also downregulated SR-A expression (Figures 2b and c). Interestingly, oxLDL, which is known to upregulate CD36,^{39,40} decreased the expression of SR-A in human monocyte-derived macrophages (Figures 2a–c) and had an additive effect when combined with insulin (Figure 2a).

Insulin and Adiponectin Decrease ABCA-1 Expression in Human Monocyte-Derived Macrophages

Immunoblots for the cholesterol transporter ABCA-1 revealed that insulin and adiponectin decreased ABCA-1 expression in macrophages by up to 80% (Figure 3a). Interestingly, PI3-kinase inhibition by LY294002 had no effect on adiponectin-mediated downregulation and only a partial effect on insulin (Figure 3a). Insulin and adiponectin also induced downregulation of ABCA-1 in the presence of oxLDL (Figure 3b), which by itself has been shown to increase ABCA-1 expression.⁴² These data suggest that the regulatory mechanism of insulin and adiponectin on ABCA-1 expression may be distinct from the liver X receptor/retinoid X receptor regulatory pathway activated by oxLDL.⁴² This is further supported by analysis of mRNA levels (Figure 3c), which showed no change in ABCA-1 levels after macrophages were exposed to insulin or adiponectin, in contrast to the 4.4-fold increase seen after exposure to oxLDL.

Insulin and Adiponectin Enhance oxLDL-Induced Lipid Accumulation in Human Monocyte-Derived Macrophages

Having shown that insulin and adiponectin increased the expression of CD36 and decreased the expression of ABCA-1 in human macrophages, we next examined their effect on oxLDL uptake, cholesterol efflux and foam cell formation. To assess oxLDL uptake, we added diI-labeled oxLDL to macrophages that had been exposed to insulin (100 nM) or adiponectin (2 μ g/ml) for 16 h and then measured intracellular fluorescence after 20 min using confocal microscopy. As shown in Figure 4a, insulin increased

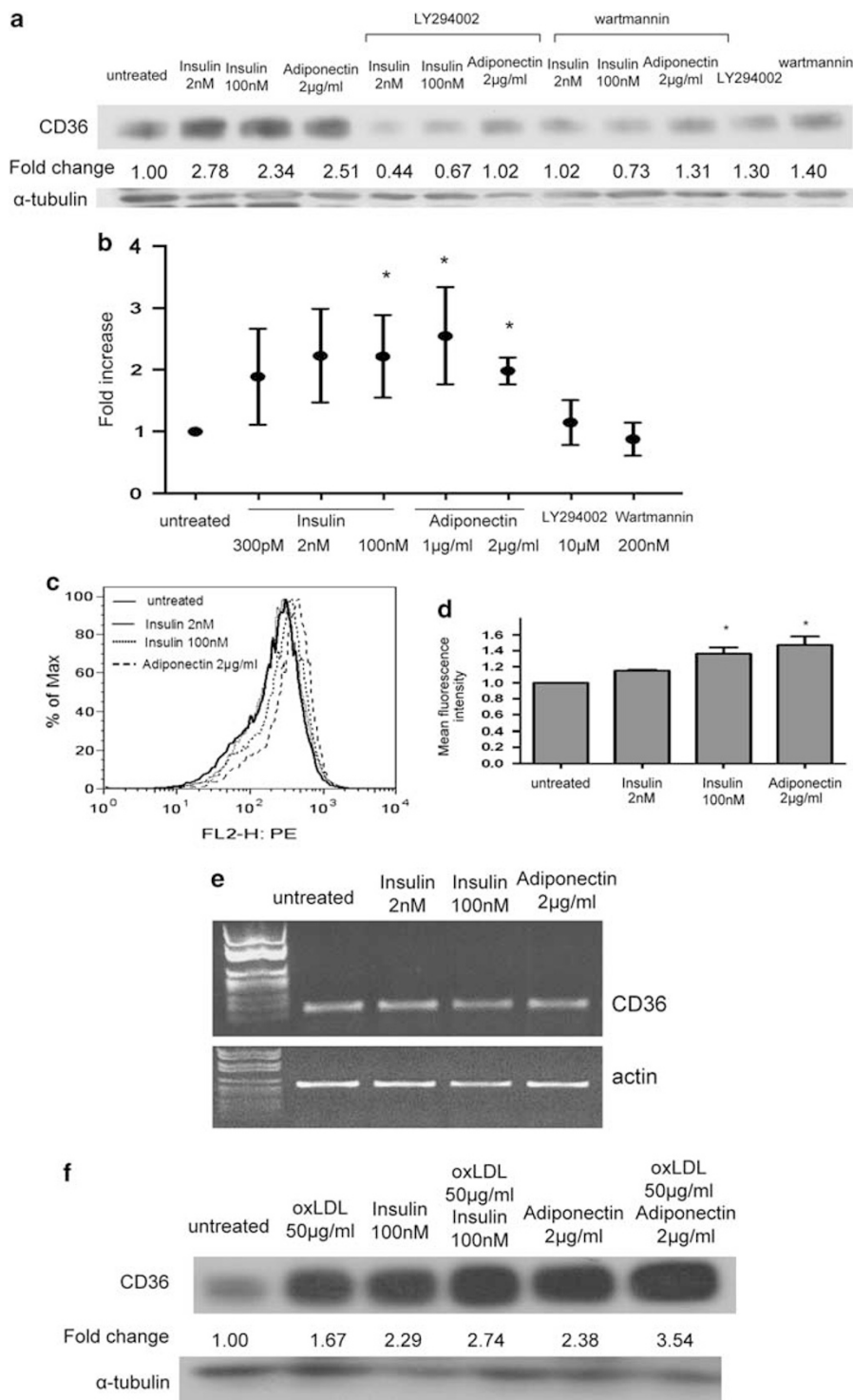


Figure 1 Insulin and adiponectin increase CD36 expression in human monocyte-derived macrophages. **(a)** Macrophages were pre-treated with indicated concentrations of insulin or adiponectin for 16 h and then lysed and assessed by western blot for CD36 expression. In some cases, cells were also treated with LY294002 (10 µM) or wartmannin (100 nM). Blots were stripped and re-probed with anti-tubulin antibody and fold change in CD36 band density was determined from scanned images. Image is representative of *n* = 15. **(b)** Means ± s.d. of data from 15 different donors normalized as in panel **a**; **P* < 0.05. **(c)** Flow cytometry histogram of cells treated with insulin or adiponectin and then stained with PE-conjugated anti-CD36 IgG. **(d)** Mean fluorescence intensity was assessed by flow cytometry as described in panel **c**. The bar graph of comparison was generated from experiments using macrophages from five different subjects; **P* < 0.001. **(e)** mRNA isolated from cells treated with insulin or adiponectin was assessed by RT-PCR using specific primers for CD36 and actin. **(f)** Macrophages pre-treated with insulin or adiponectin along with or without oxidized LDL (oxLDL) were assessed by western blot for CD36 expression as described in panel **a**.

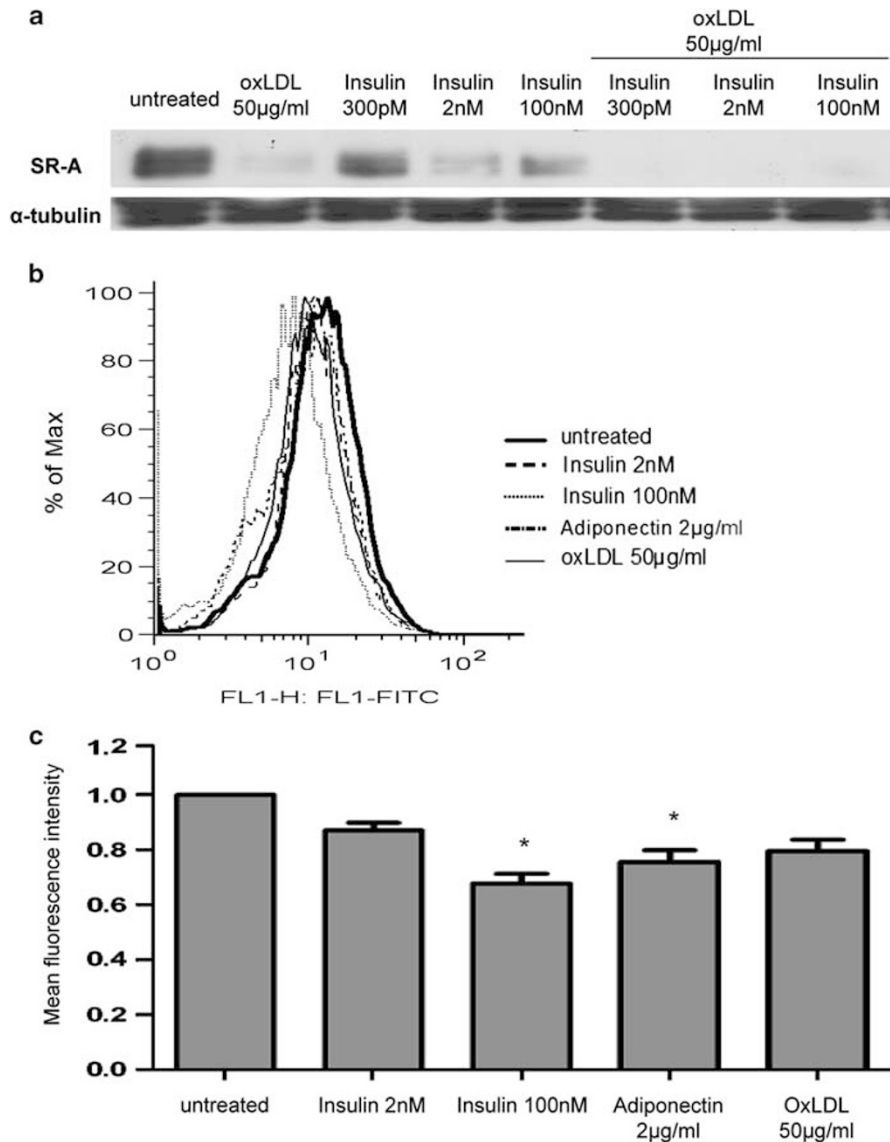


Figure 2 Insulin and adiponectin decrease expression of scavenger receptor-A (SR-A) in human monocyte-derived macrophages. Macrophages were pre-treated with insulin, adiponectin, oxidized LDL (oxLDL) or insulin plus oxLDL for 16 h and analyzed by immunoblot (**a**) or flow cytometry (**b**) as in Figure 1 using a monoclonal antibody specific for SR-A. Experiments were repeated with macrophages from three different donors, respectively. (**c**) Mean fluorescence intensity was assessed by flow cytometry in panel **b**. The bar graph of comparison was generated from experiments using macrophages from three different subjects; * $P < 0.05$.

uptake by $\sim 80\%$ ($P < 0.05$) whereas adiponectin had no effect (Figure 4a).

To evaluate the effect of insulin and adiponectin on cholesterol efflux from macrophages, we loaded cells with oxLDL-containing C^{14} -labeled cholesterol and then measured the rate of C^{14} efflux to apoA1 in the culture medium. Pre-treatment with either insulin or adiponectin decreased the efflux by $\sim 25\%$ (Figure 4b; $P < 0.05$).

We hypothesized that the increased macrophage uptake of oxLDL and impaired cholesterol efflux induced by insulin or adiponectin would result in intracellular accumulation of excessive lipoprotein derived cholesterol and ultimately in

foam cell formation.⁴³ Figure 4c shows that after 16 h of cell exposure to oxLDL, intracellular cholesterol content measured by enzymatic cholesterol assay was increased by threefold in macrophages pre-treated with insulin or adiponectin, compared with untreated cells. Total cholesterol and free cholesterol of macrophages measured by GC-MS also showed that free and total intracellular cholesterol of macrophages were increased by pre-treatment with insulin or adiponectin (Figures 4d and e).

OxLDL is known to induce secretion of cytokines such as MCP-1 and IL-6 from mouse macrophages.⁴⁴ Here we show that oxLDL also increased the secretion of IL-6 and MCP-1

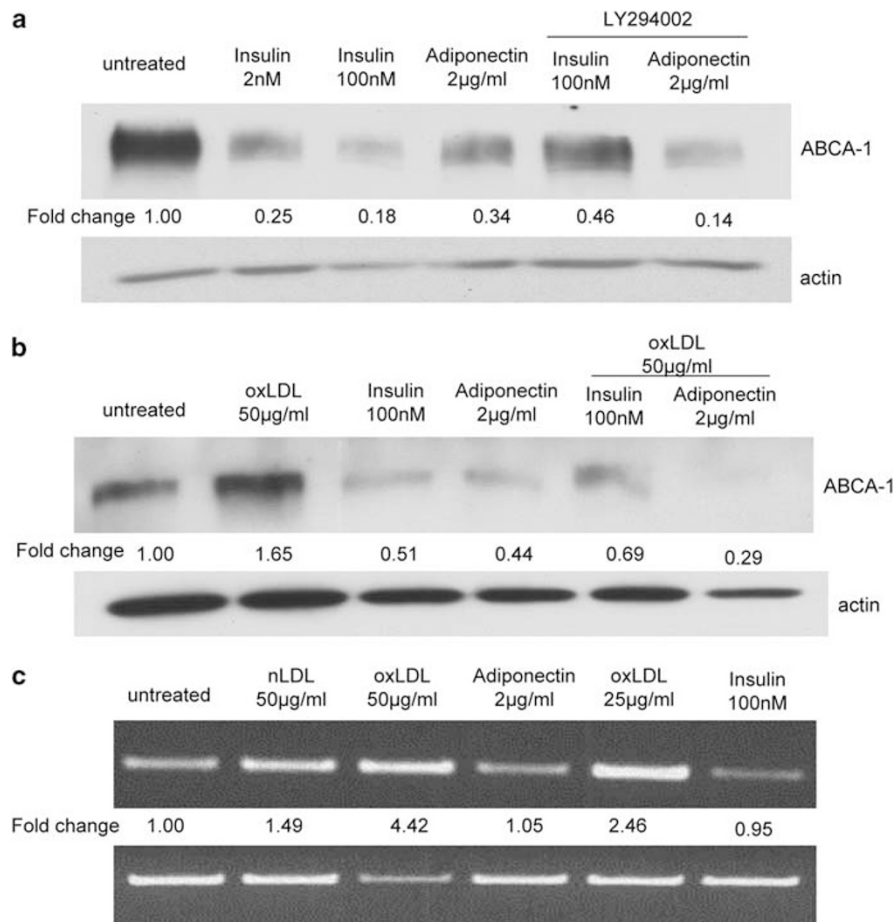


Figure 3 Insulin and adiponectin decrease ATP-binding cassette transporter sub-family A member 1 (ABCA-1) expression in human monocyte-derived macrophages. **(a)** Macrophages were pre-treated for 16 h with insulin or adiponectin in the presence or absence of LY294002 (10 µM) and then lysed and analyzed by immunoblot for ABCA-1 expression. Blots were stripped and re-probed with anti-actin antibody and fold change in ABCA-1 band density was determined from scanned images. Image is representative of three repetitive blots. **(b)** Cells were exposed to oxidized LDL (oxLDL; 50 µg/ml) with or without insulin or adiponectin and analyzed as in panel **a**. **(c)** RT-PCR for ABCA-1 mRNA of macrophages treated as described in panel **b**.

from human monocyte-derived macrophages (Supplementary Figure 1), and that neither insulin nor adiponectin affected baseline or oxLDL-induced cytokine secretion.

DISCUSSION

Hyperinsulinemia was first suggested as a risk factor for atherosclerosis more than 30 years ago, based on the observation that insulin levels are higher than normal in patients with ischemic heart disease.⁴⁵ Since then, there have been many clinical and experimental studies revealing that high levels of insulin precede development of arterial diseases in diabetic and non-diabetic patients.^{12–17,46–48} However, the role of hyperinsulinemia as an independent risk factor for atherosclerotic coronary disease has been controversial. Mostly, hyperinsulinemia occurs with insulin resistance in type 2 diabetes and is regarded as a compensatory mechanism of insulin resistance. Many studies suggest insulin resistance as a risk factor for atherosclerosis based on its pathological effects on dyslipidemia, hypertension, and a

hypercoagulable state, which accelerate atherosclerosis.^{6–11,49} Therefore, it is difficult to determine whether the link between hyperinsulinemia and atherosclerosis is causative, and this compels more experimental studies.

Macrophages perform a crucial role in the atherogenic process by generating lipid laden foam cells.²⁰ Macrophages are known to express most insulin signaling molecules except IR substrate 1 (IRS1) and glucose transporter type 4.^{50,51} Even though insulin activates the IR/IRS2/PI3K/Akt pathway in macrophages as in other types of insulin-responsive cells, there have been few studies investigating the biological functions of insulin signaling in macrophages. In the current study, we evaluated if insulin affects macrophage foam cell formation and found that insulin increased the expression of CD36 and decreased ABCA-1 expression, which may promote cholesterol accumulation in human monocyte-derived macrophages. Although the mechanism is not clear, the insulin-mediated regulatory mechanism of CD36 and ABCA-1 appear to be post-transcriptional based on our results from

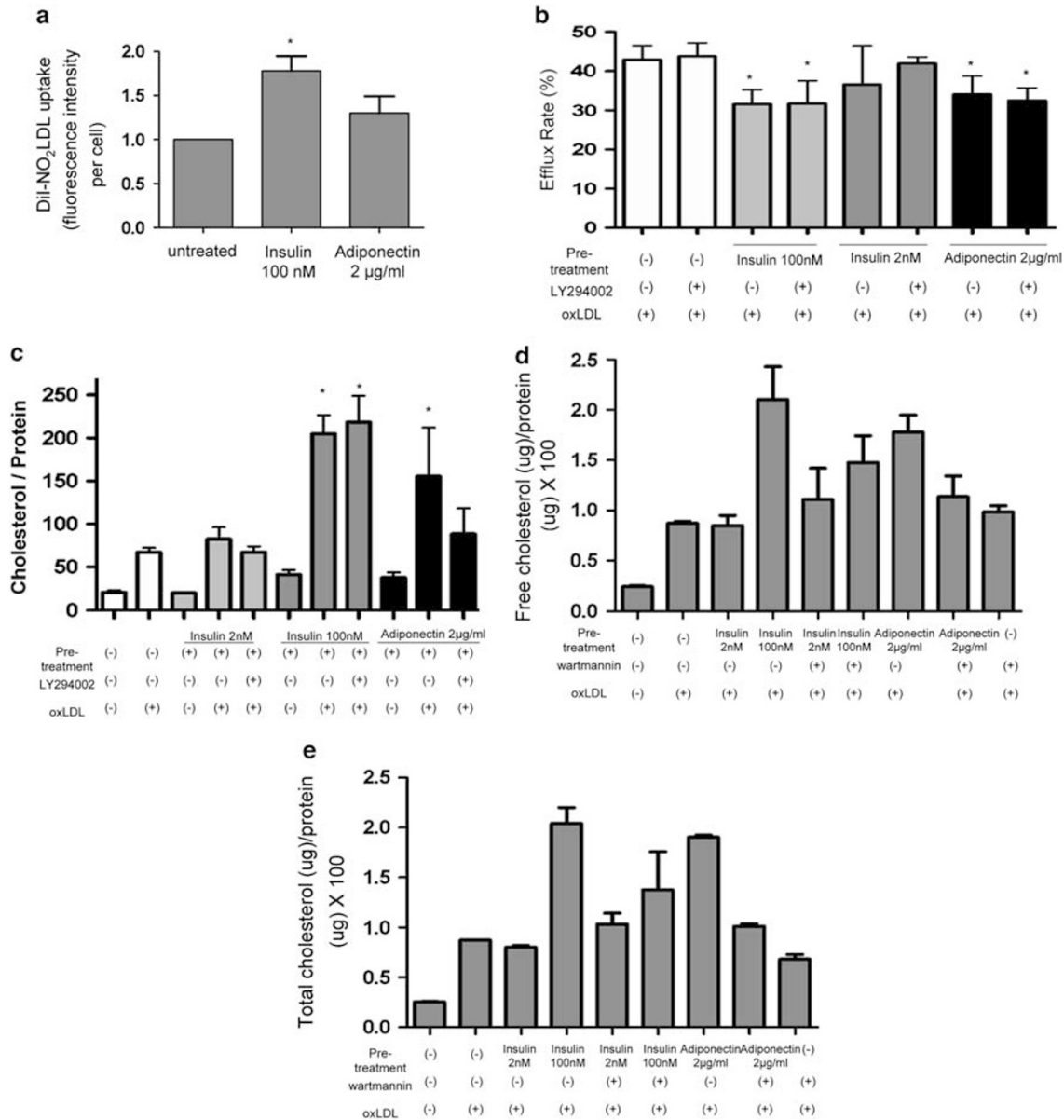


Figure 4 Insulin and adiponectin enhance oxidized LDL (oxLDL)-mediated cholesterol loading of human monocyte-derived macrophages. (a) Macrophages were pre-treated with insulin (100 nM) or adiponectin (2 μg/ml) for 16 h and then exposed to 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanide perchlorate (dii)-labeled oxLDL for 20 min at 37°C. Fluorescence uptake was quantified by digital confocal microscopy using Imge-Pro software. The graph represents mean ± s.d. of five experiments analyzing 50–100 cells each (**P* < 0.05). (b) Macrophages were pre-treated with insulin or adiponectin for 16 h and then exposed to oxLDL-containing C¹⁴-labeled cholesterol for 6 h. C¹⁴-labeled cholesterol effluxed to apolipoprotein A1 (apoA1) was measured by scintillation counting. The graph shows mean ± s.d. from separate assays using macrophages from three different donors (**P* < 0.05). (c) Macrophages were pre-treated with insulin or adiponectin as above and then exposed to oxLDL (50 μg/ml) for 16 h at 37°C. Intracellular cholesterol was measured by the cholesterol oxidation reaction method. The graph shows mean ± s.d. from experiments using macrophages from three different donors (**P* < 0.001). Macrophages pre-treated with insulin or adiponectin were exposed to oxLDL (50 μg/ml). Free (d) or total (e) cholesterol was measured by gas chromatography combined with mass spectrometry (GC-MS).

RT-PCR. A recent study showing that insulin increased CD36 expression in Chinese hamster ovary or HEK 293 cells via regulating CD36 turnover⁵² supports our observation and permits a possible expectation that a similar regulatory pathway may be activated in macrophages. The mechanisms of insulin regulation of CD36 and ABCA-1 need to be studied

and it appears to be that these proteins are regulated via different pathways.

Adiponectin is known to enhance insulin sensitivity, however, the signaling mechanism by which adiponectin sensitizes insulin is not clear. In our study, adiponectin showed an overlapping signaling with insulin. Low concentration of

adiponectin increased the phosphorylation of Akt (Ser436) by the same degree as insulin and had the same modulating effect on CD36 and ABCA-1 as insulin (Figures 1 and 3). Previous studies showed that adiponectin had an anti-atherogenic property in apolipoprotein E-deficient mice⁵³ and one of the suggested mechanisms was an inhibitory effect of adiponectin on SR-A expression and acetylated LDL (acLDL) uptake.⁵⁴ In our current study, we reproduced the previous finding and showed that both insulin and adiponectin decreased SR-A expression in macrophages. However, the net effect of adiponectin resulted in increased intracellular cholesterol accumulation, which was opposite of previous reports. In addition to SR-A, adiponectin is known to downregulate ACAT-1 that catalyzes cholesteryl ester (CE) formation³³ and therefore, adiponectin treatment decreased acLDL-induced CE accumulation in macrophages.^{33,55} These intriguing results may be due to the different sources of cholesterol for lipid uptake assays, different concentrations of adiponectin and different modes of adiponectin activities. In our study using MPO-modified LDL (oxLDL), which is known to be a specific ligand for CD36,⁵⁶ adiponectin increased the uptake of oxLDL via increased expression of CD36, whereas in the previous studies performed with acLDL, a specific ligand for SR-A,⁵⁷ adiponectin, decreased acLDL uptake via a decrease in SR-A. Therefore, the effect of adiponectin *in vivo* may be determined by the specificity of modified LDL for different scavenger receptors.

Changes in the protein levels of CD36 and ABCA-1 induced by insulin and adiponectin appear to be regulated by PI3-kinase. Our study showed that PI3-kinase inhibitor prevented the increase of CD36 expression induced by insulin and adiponectin (Figure 1) while it minimally blocked the effect of these reagents on the expression of ABCA-1 (Figure 3). The functional effect of PI3-kinase blockade on the intracellular cholesterol of macrophages was varied among macrophages from different donors (Figure 4). As expected, based on the minimal blockade of ABCA-1 decrease, the PI-3 kinase inhibitor had no effect on cholesterol efflux of macrophages (Figure 4b) but partially prevented the increase of intracellular cholesterol by blocking increased CD36 expression.

Atherosclerosis and type 2 diabetes share similar pathological mechanisms including elevation in cytokines like MCP-1 and interleukin-6 (IL-6), which contribute to underlying inflammation of both.⁵⁸ OxLDL is abundant in both of these conditions and is known to induce secretion of these proinflammatory cytokines in macrophages.⁵⁹ Previous studies have suggested anti-inflammatory activities of insulin by showing that insulin infusion to diabetic patients suppressed mononuclear cell expression of toll-like receptor (TLR)-2 and TLR-4.⁶⁰ However, another study showed that prolonged exposure to insulin accentuated tumor necrosis factor- α -induced transcription of proinflammatory genes while short-term exposure inhibited the transcription.⁶¹ In our current study, insulin and adiponectin, also known to have anti-in-

flammatory activity,⁶² did not affect oxLDL-induced secretion of MCP-1 and IL-6 in macrophages.

In the current study, we propose evidence that insulin facilitates macrophage foam cell formation, although this is a topic of controversy. It is sometimes suggested that hyperinsulinemia in the presence of insulin resistance may not be metabolically effective, however, it is possible that one pathway may remain active when the other pathway is blocked by insulin resistance. Therefore, more studies are needed to show how insulin activates different pathways and may be involved in different biological functions specifically affected by insulin resistance. The risk of cardiovascular disease is 10-fold higher than normal in patients with type 1 diabetes.⁶³ Even though type 1 diabetes is characterized by impaired insulin secretion, it does not rule out the possible pathological effect of hyperinsulinemia. Indeed, many patients with type 1 diabetes have hyperinsulinemia from excessive dose of insulin, resulting insulin resistance.⁶⁴ Furthermore, hyperinsulinemia appears to drive insulin resistance.⁶⁵ Therefore, for the proper management of patients with type 1 and type 2 diabetes, more investigation about the role of hyperinsulinemia is required.

In conclusion, we provide evidence that hyperinsulinemia may promote atherosclerosis by promoting macrophage foam cell formation in the setting of abundant oxLDL, which has specific affinity to CD36.

Supplementary Information accompanies the paper on the Laboratory Investigation website (<http://www.laboratoryinvestigation.org>)

ACKNOWLEDGEMENTS

We are grateful to David Schmitt, Dr Xin-Min Li and Robert Koeth in Dr Stanley Hazen's laboratory at the Cleveland Clinic for helping us perform the cholesterol efflux assay and GC-MS. This study was supported by NIH P01 HL087018 (RLS) and a KL2 award (SK) from the Case Western Reserve University Clinical Translational Research Award UL1RR024989.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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