REFERENCES

Ashby, D. T., Rye, K.-A., Clay, M. A., Vadas, M. A., Gamble, J. R., Barter, P. J. Factors influencing the ability of HDL to inhibit expression of vascular cell adhesion molecule-1 in endothelial cells. Arterioscler Thromb Vasc Biol. 18 (1998): 1450-5.

Ashby, D., Gamble, J., Vadas, M., Fidge, N., Siggins, S., Rye, K.-A. et al. Lack of effect of serum amyloid A (SAA) on the ability of high-density lipoproteins to inhibit endothelial cell adhesion molecule expression. Atherosclerosis. 154 (2001): 113-21.

Baker, P. W., Rye, K.-A., Gamble, J. R., Vadas, M. A., Barter, P. J. Ability of reconstituted high density lipoproteins to inhibit cytokine-induced expression of vascular cell adhesion molecule-1 in human umbilical vein endothelial cells. J. Lipid Res. 40 (1999): 345-53.

Barlage, S., Frohlich, D., Bottcher, A., Jauhiainen, M., Muller, H. P., Noetzel,F. et al. ApoE-containing high density lipoproteins and phospholipids transfer protein activity increase in patients with a systemic inflammatory response. J. Lipid Res. 42 (2001): 281-90.

Barter, P. Effects of inflammation on high-density lipoproteins. Arterioscler. Thromb. Vasc. Biol. 22 (2002): 1062-3.

Beveridge, T. J. Structures of gram-negative cell walls and their derived membrane vesicles. J. Bacteriol. 181(16) (1999): 4725-33.

Brandenburg, K., Jurgens, G., Andra, J., Lindner, B., Koch, M. H. J., Blume, A. et al. Biophysical characterization of the interaction of high - density lipoprotein (HDL) with endotoxins. Eur. J. Biochem. 269 (2002): 5972-81.

Brito, G. A. C., Falcao, J. L. A. A., Saraiva, S. N. R., Lima, A. A. M., Flores, C. A., and Ribeiro, R. A. Histopathological analysis of rat mesentery as a method for evaluating neutrophil migration: differential effects of dexamethasone and pertussis toxin. Braz. J. Med. Biol. Res. 31 (1998): 1319-27.

Cabana, V. G., Siegel, J. N., Sabesin, S. M. Effects of the acute phase response on the concentration and density distribution of plasma lipids and apolipoproteins. J. Lipid Res. 30 (1989): 39–49.

Cavaillon, J. M., Fitting, C., Haeffner-Cavaillon, N., Kirsch, S. J., Warren, H. S. Cytokine response by monocytes and macrophages to free and lipoprotein-bound lipopolysaccharide. Infect. Immun. 58(7) (1990): 2375-82.

Chakraphan, D., Sridulyakul, P., Thipakorn, B., Bunnag, S., Huxley, V. H., Patumraj, S. Attenuation of endothelial dysfunction by exercise training in STZ-induced diabetic rats. Clin. Hemorheol. Microcirc. 32(3) (2005): 217-26.

Chow, J. C., Young, D. W., Golenbock, D. T., Christ, W. J., Gusovsky, F. Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. J. Biol. Chem. 274(16) (1999): 10689-92.

Cockerill, G. W., Rye, K.-A., Gamble, J. R., Vadas, M. A., Barter, P. J. High-density lipoproteins inhibit cytokine-induced expression of endothelial cell adhesion molecules. Arterioscler Thromb Vasc Biol. 15 (1995): 1987-1994.

Cockerill, G. W., Huehns, T. Y., Weerasinghe, A., Stocker, C., Lerch, P. G., Miller, N. E. et al. Elevation of plasma high-density lipoprotein concentration reduces interleukin-1-induced expression of E-selectin in an in vivo model of acute inflammation. Circulation. 103 (2001): 108-112.

Davenpeck, K. L., Zagorski, J., Schleimer, R. P., Bochner, B. S. Lipopolysaccharide-induced leukocyte rolling and adhesion in the rat mesenteric microcirculation : regulation by glucocorticoids and role of cytokines. J. Immunol. 161 (1998): 6861-70.

Dentener, M. A., Bazil, V., Von Asmuth, E. J. U., Ceska, M., Buurman, W. A. Involvement of CD14 in lipopolysaccharide-induced tumor necrosis factor-α, IL-6 and IL-8 release by human monocytes and alveolar macrophages. J. Immunol. 150 (1993): 2885-91.

Ehrenwald, E., Chisolm, G. M., Fox, P. L. Intact human ceruloplasmin oxidatively modifies low density lipoprotein. J. Clin. Invest. 93 (1994): 1493-501.

Emancipator, K., Csako, G., and Elin, R. J. *In vitro* inactivation of bacterial endotoxin by human lipoproteins and apolipoproteins. Infect. Immun. 60 (1992): 596-601.

Epstein, F. H. Acute-phase proteins and other systemic responses to inflammation. N. Engl. J. Med. 340(6) (1999): 448-54.

Feingold, K. R., Hardardottir, I., Memon, R., Krul, E. J., Moser, A. H., Taylor, J. M., and Grunfeld, C. Effect of endotoxin on cholesterol biosynthesis and distribution in serum lipoproteins in Syrian hamsters. J. Lipid Res. 34 (1993): 2147–58.

Feingold, K. R., Funk, J. L., Moser, A. H., Shigenaga, J. K., Rapp, J. H., Grunfeld, C. Role for circulating lipoproteins in protection from endotoxin toxicity. Infect. Immun. 63 (1995): 2041-6.

Flegel, W. A., Baumstark, M. W., Weinstock, C., berg, A., and Northhoff, H. Prevention of endotoxin-induced monokine release by human low- and high-density lipoproteins and by apolipoprotein A-I. Infect. Immun. 61 (1993): 5140-6.

Gabay, C., and Kushner, I. Acute-phase proteins and other systemic responses to inflammation. N. Engl. J. Med. 340 (1999): 448-54.

Gavins, F. N. E., Chatterjee, B. E. Intravital microscopy for the study of mouse microcirculation in anti-inflammatory drug research: Focus on the mesentery and cremaster preparations. J. Pharm. Toxic. Meth. 49 (2004): 1-14.

Gerhardt, P., and Drew, S. W. Liquid culture. In Gerhardt, Editor, Methods for general and molecular bacteriology, pp. 228. USA: American Society for Microbiology, 1994.

Grunfeld, C., Pang, M., Doerrler, W., Shigenaga, J. K., Jensen, P., and Feingold, K. R. Lipids, lipoproteins, triglyceride clearance, and cytokines in haman immunodeficiency virus infection and the acquired immunodeficiency syndrome. J. Clin. Endocrinol. Metab. 74 (1992): 1045-52.

Grunfeld, C., Marshall, M., Shigenaga, J. K., Moser, A. H., Tobias, P., and Feingold, K. R. Lipoproteins inhibit macrophage activation by lipoteichoic acid. J. Lipid Res. 40 (1999): 245-52.

Gupta, H., Dai, L., Datta, G., Garber, D. W., Grenett, H., Li, Y. et al. Inhibition of lipopolysaccharide-induced inflammatory responses by an apolipoprotein AI mimetic peptide. Circ. Res. 97 (2005): 236-43.

Gutsmann, T., Muller, M., Carroll, S. F., MacKenzie, R. C., Wiese, A., and Seydel, U. Dual role of lipopolysaccharide (LPS)-binding protein in neutralization of LPS and enhancement of LPS-induced activation of mononuclear cells. Infect. Immun. 69 (2001): 6942-50.

Hailman, E., Albers, J. J., Wolfbauer, G., Tu, A. Y., Wright, S. D. Neutralization and transfer of lipopolysaccharide by phospholipids transfer protein. J. Biol. Chem. 271(21) (1996): 12172-8.

Hamann, L., Alexander, C., Stamme, C., Zahringer, U., and Schumann, R. R. Acute-phase concentrations of lipopolysaccharide (LPS)-binding protein inhibit innate immune cell activation by different LPS chemotypes via different mechanisms. Infect. Immun. 73(1) (2005): 193-200.

Havel, R. J., Eder, H. A., Bragdon, J. H. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J. Clin. Invest. 34(9) (1955): 1345-53.

Haziot, A., Rong, G. W., Silver, J., Goyert, S. M. Recombinant soluble CD14 mediates the activation of endothelial cells by lipopolysaccharide. J. Immunol. 151(3) (1993): 1500-7.

Heckly, R. J. Preservation of microorganisms. Adv. Appl. Microbiol. 24 (1978): 1-53.

Hoffman, J. S., and Benditt, E. P. Changes in high density lipoprotein content following endotoxin administration in the mouse : formation of serum amyloid protein-rich subfractions. J. Biol. Chem. 257(17) (1982):10510–7.

Hyka, N., Dayer, J.-M., Modoux, C., Kohno, T.,Edwards III, C. K., Roux-Lombard, P. et al. Apolipoprotein A-I inhibits the production of interleukin-1β and tumor necrosis factor-α by blocking contact-mediated activationof monocytes by T lymphocytes. Blood. 97 (2001): 2381-9.

Ishida, I., Kubo, H., Suzuki, S., Suzuki, T., Akashi, S., Inoue, K. et al. Hypoxia diminishes toll-like receptor 4 expression through reactive oxygen species generated by mitochondria in endothelial cells. J. Immunol. 169 (2002): 2069-75.

Jiang, Q., Akashi, S., Miyake, K., Petty, H. R. Cutting edge : lipopolysaccharide induces physical proximity between CD14 and toll-like receptor 4 (TLR4) prior to nuclear translocation of NF-κB. J. Immunol. 165 (2000): 3541-4.

Juan, M. A., Xue-Ling, L., Bin, L., and Man-Ping,W. U. Role of apolipoprotein A-I in protecting against endotoxin toxicity. Acta. Biochim. Biophys. Sin. 36(6) (2004): 419-24.

Khovidhunkit, W., Memon, R. A., Feingold, K. R., and Grunfeld, C. Infection and inflammation-induced proatherogenic changes of lipoproteins. J. Infect. Dis. 181 (2000):S462-72.

Khovidhunkit, W., Shigenaga, J. K., Moser, A. H., Feingold, K. R., and Grunfeld, C. Cholesterol efflux by acute-phase high density lipoprotein: role of lecithin: cholesterol acyltransferase. J. Lipid Res. 42 (2001):967-75.

Khovidhunkit, W., Kim, M. S., Memon, R. A., Shigenaga, J. K., Moser, A. H., Feingold, K. R., Grunfeld, C. Effects of infection and inflammation on lipid and lipoprotein metabolism : mechanisms and consequences to the host. J. Lipid Res. 45 (2004): 1169-96.

Khovidhunkit, W.,Hachem, J. P., Medzihradszky, K. F., Duchateau, P. N., Shigenaga, J. K., Moser, A. H. et al. Parotid secretory protein is an HDL-associated protein with anticandidal activity. Am. J. Physiol. Regul. Integr. Comp. Physiol. 288 (2005): 1306-15.

Koch, A. Growth measurement. In Gerhardt, Editor, Methods for General and Molecular Bacteriology, pp. 254-7. USA: American Society for Microbiology, 1994.

Koneman, E. W., Allen, S. D., Janda, W. M., Schreckenbergre, P. C., and Winn, W. C. Basic bacteriology, concepts of virulence, and technologic advances in clinical microbiology : an overview. In Color Atlas and Textbook of Diagnostic Microbiology, Fifth edition, pp. 1-67. New York: Lippincott. Philadelphia, 1997.

Laemmli, U. K. Cleavage of structure proteins during the assembly of the head of bacteriophage T4. Nature. 227 (1970): 680-5.

Lamping, N., Dettmer, R., Schroder, N. W. J., Pfeil, D., Hallatschek, W., Burger, R. et al. LPS-binding protein protects mice from septic shock caused by LPS or gram-negative bacteria. J. Clin. Invest. 101(10) (1998): 2065-71.

Levels, J. H. M., Abraham, P. R., Van den Ende, A., Van Deventer, S. J. H. Distribution and kinetics of lipoprotein-bound endotoxin. Infect. Immun. 69 (2001): 2821-8.

Levine, D. M., Parker, T. S., Donnelly, T. M., Walsh, A., and Rubin, A. L. *In vivo* protection against endotoxin by plasma high-density lipoprotein. Proc Natl. Acad. Sci U.S.A. 90 (1993): 12040-44.

Ly, H., Omar, L. F., Christopher, J. F., Judy, K. S., Arther, H. M., Carl, G. et al. Endotoxin and TNF lead to reduced plasma LCAT activity and decreased hepatic LCAT mRNA levels in syrian hamsters. J. Lipid Res. 36 (1995): 1254-63.

Madigan, M. T., Martinko, J. M., Parker, J. Biology of Microorganisms. Ninth edition. USA.: Prentice Hall international, Inc., 2000.

Mantovani, A., Dejana, E. Cytokines as communication signals between leukocytes and endothelial cells. Immunol. Today. 10(11) (1989): 370-5.

Memon, R. A., Staprans, I., Noor, M., Holleran, W. M., Uchida, Y., Moser, A. H. et al. Infection and inflammation induce LDL oxidation *in vivo*. Arterioscler Thromb Vasc Biol. 20 (2000): 1536-42.

Munford, R. S., Hall, C. L., Dietschy, J. M. Binding of Salmonella typhimurium lipopolysaccharides to rat high-density lipoproteins. Infect. Immun. 34 (1981): 835-43.

Muzio, M., Polentarutti, N., Bosisio, D., Manoj Kumar, P. P., Mantovani, A., Toll-like receptor family and signaling pathway. Biochem. Soc. Trans. 28(5) (2000): 563-6.

Nicholls, S. J., Dusting, G. J., Cutri, B., Bao, S., Drummond, G. R., Rye, K.-A. et al. Reconstituted high-density lipoproteins inhibit the acute pro-oxidant and proinflammatory vascular changes induced by a periarterial collar in normocholesterolemic rabbits. Circulation. 111 (2005): 1543-50.

Pajkrt, D., Doran, J. E., Koster, F., Lerch, P. G., Arnet, B., Van der Poll, T., Ten Cate, J. W., and Van Deventer, S. J. Antiinflammatory effects of reconstituted high-density lipoprotein during human endotoxemia. J. Exp. Med. 184 (1996): 1601-8.

Parker, T. S., Levine, D. M., Chang, J. C. C., Baxter, J., Coffin, C. C., Rubin, A. L. Reconstituted high-density lipoprotein neutralizes gram-negative bacterial lipopolysaccharides in human whole blood. Infect. Immun. 63 (1995): 253-8.

Perez-Llamas, F., Zamora, S., Rosique, M. J., and Sastre, J. F. Effects of inhalation of ethyl-ether on glycemia and on some variables of intermediate metabolism in rats. Arch. Int. Physiol. Biochim. Biophys. 100(5) (1992): 335-7.

Perez-Morga, D., Vanhollebeke, B., Paturiaux-Hanocq, F., Nolan, D. P., Lins, L., Homble, F. et al. Apolipoprotein L-I promotes Trypanosome lysis by forming pores in lysosomal membranes. Science. 309(5733) (2005): 469-72.

Pugin, J., Schurer-Maly, C. C., Leturcq, D., Moriarty, A., Ulevitch, R. J., Tobias, P. S. Lipopolysaccharide activation of human endothelial andepithelial cells is mediated by lipopolysaccharide-binding protein and soluble CD14. Proc. Natl. Acad. Sci. USA. 90 (1993): 2744-8.

Pugin, J., Ulevitch, R. J., Tobias, P. S. A critical role for monocytes and CD14 in endotoxin-induced endothelial cell activation. J. Exp. Med. 178 (1993): 2193-200.

Read, T. E., Grunfeld, C., Kumwenda, Z. L., Calhoun, M. C., Kane, J. P., Feingold, K. R. Triglyceride-rich lipoproteins prevent septic death in rats. J. Exp. Med. 182(1995): 267-72.

Rifkin, M. R. Role of phospholipids in the cytotoxic action of high density lipoprotein on trypanosomes. J. Lipid Res. 32 (1991): 639-47.

Schletter, J., Heine, H., Ulmer, A. J., Rietscel, E. T. Molecular mechanisms of endotoxin activity. Arch. Microbiol. 164 (1995): 383-9.

Smith, R. P., Baltch, A. L., Michelsen, P. B., Ritz, W. J., and Alteri, R. Use of the microbial growth curve in postantibiotic effect studies of *Lgeionella pneumophila.* Antimicrob. Agents Chemother. 47(3) (2003): 1081-7.

Sperry, W. M., Brand, F. C. The determination of total lipids in blood serum. J. Biol. Chem. 213 (1955): 69-76.

Sprong, T., Netea, M. G., Van der Ley, P., Verver-Jansen, T. J. G., Jacobs, L. E. H., Stalenhoef, A. et al. Human lipoproteins have divergent neutralizing effects on *E. coli* LPS, *N. meningitides* LPS, and complete gram-negative bacteria. J. Lipid Res. 45 (2004): 742-9.

Stannard, A. K., Khan, S., Graham, A., Owen, J. S., Allen, S. P. Inability of plasma high-density lipoproteins to inhibit cell adhesion mocule expression in human coronary artery endothelial cells. Atherosclerosis. 154 (2001): 31-8.

Tada, N., Sakamoto, T., Kagami, A., Mochizuki, K., and Kurosaka, K. Antimicrobial activity of lipoprotein particles containing apolipoprotein A-I. Mol. Cell. Biochem. 119 (1993): 171-8.

Tall, A. R. Plasma lipid transfer proteins. J. Lipid Res. 27 (1986): 361-7.

Tuck, C. H. Disorders of lipid metabolism. In Abrahamson, M., editor, Educational Review Manual in Endocrinology, Diabetes, and

Metabolism. Second Edition, pp. 1-9. New York: Castle Connolly Graduate Medical Publishing, Limited., 2002.

Ulevitch, R. J., and Johnston, A. R. The modification of biophysical and endotoxic properties of bacterial lipopolysaccharides by serum. J. Clin. Invest. 62 (1978): 1313-24.

Ulevitch, R. J., Johnston, A. R., and Weinstein, D. B. New function for high density lipoproteins : their participation in intravascular reactions of bacterial lipopolysaccharides. J. Clin. Invest. 64 (1979): 1516-24.

Ulevitch, R. J., Johnsto, A. R., and Weinstein, D. B. New function for high density lipoproteins : isolation and characterization of a bacterial lipopolysaccharide-high density lipoprotein complex formed in rabbit plasma. J. Clin. Invest. 67 (1981): 827-37.

Van Lenten, B. J., Hama, S. Y., de Beer, F. C. et al. Anti-inflammatory HDL becomes pro-inflammatory during the acute phase response. Loss of protective effect of HDL against LDL oxidation in aortic wall cell co-cultures. J. Clin. Invest. 96 (1995): 2758-67.

Van Oosten, M., Rensen, P. C. N., Van Amersfoort, E. S., Van Eck, M., Van Dam, A.-M., Breve J. J. P. et al. Apolipoprotein E protects against bacterial lipopolysaccharide-induced lethality. J. Biol. Chem. 276(2) (2001): 8820-4.

Weinstein, D. B., and Ulevitch, R. J. A new protective role for high density lipoprotein (HDL): detoxification of endotoxins. Circulation. 58(Suppl. II) (1979): II-90 (Abstr.).

Woodman, R. C., Teoh, D., Payne, D., Kubes, P. Thrombin and leukocyte recruitment in endotoxemia. Am. J. Physiol. Heart. Circ. Physiol. 279 (2000): 1338-45.

Woollett, L. A., Spady, D. K. Kinetic parameters for high density lipoprotein apolipoprotein A-l and cholesteryl ester transport in the hamster. J. Clin. Invest. 99 (1997): 1704-13.

Wurfel, M. M., Kunitake, S. T., Lichenstein, H., Kane, J. P., Wright, S. D. Lipopolysaccharide (LPS)-binding protein is carried on lipoproteins and acts as a cofactor in the neutralization of LPS. J. Exp. Med. 180 (1994): 1025-35.

Wurfel, M. M. and Wright, S. D. Lipopolysaccharide-binding protein and soluble CD14 transfer lipopolysaccharide to phospholipids bilayers. J. Immunol. 258 (1997): 3925-34.

Yipp, B. G., Andonegui, G., Howlett, C. J., Robbins, S. M., Hartung, T., Ho, M. et al. Profound differences in leukocyte-endothelial cell responses to lipopolysaccharide versus lipoteichoic acid. J. Immunol. 168 (2002): 4650-8.

APPENDICES

APPENDIX I

PUBLICATIONS

Krishnamra, N. and Taweerathitam, P. Acute effect of prolactin on active calcium absorption in rats. Can. J. Physiol. Pharmacol. 73(8) (1995): 1185 – 9.

Thaveeratitham, P., Khovidhunkit, W., Patumraj, S. High – density lipoproteins (HDL) inhibit endotoxin-induced leukocyte adhesion on endothelium in rats : effect of the acute-phase HDL. Clin. Hemorheol. Microcirc. (submitted).

High-density lipoproteins (HDL) inhibit endotoxin-induced leukocyte adhesion on endothelial cells in rats : effect of the acute-phase HDL

Premtip Thaveeratitham[a], Weerapan Khovidhunkit[b] and Suthiluk Patumraj[c,*]

[a]Inter-Department of Physiology, Graduate School, Chulalongkorn University, Bangkok 10330, Thailand.

[b]Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

[c]Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

*Corresponding author: Assoc. Prof. Suthiluk Patumraj, Ph.D

Department of Physiology

Faculty of Medicine, Chulalongkorn University

Physiological building

King Chulalongkorn Memorial Hospital

Patumwan, Bangkok 10330, Thailand

Phone: 011-662-256-4267

Fax: 011-662-252-7854

E-mail: medspr@hotmail.com

Abstract:

High-density lipoprotein (HDL) plays an important role not only in protecting against atherosclerosis but also in innate immunity. Several lines of evidence has shown that HDL could ameliorate the toxic effects of endotoxin or lipopolysaccharide (LPS). In this study, we examined whether HDL could inhibit LPS-induced leukocyte adhesion on endothelial cells. Normal HDL and acute-phase HDL (AP-HDL) were purified from plasma of hamsters that received normal saline and LPS injection, respectively. Wistar rats were given LPS injection and the number of leukocytes adhered on endothelial cells of the mesenteric venules were determined using intravital fluorescence microscopy. Intravenous injection of LPS enhanced leukocyte adhesion to the mesenteric venules. However, when LPS was preincubated with normal HDL, leukocyte adhesion on endothelial cells in response to LPS was significantly attenuated in a dose-dependent manner. AP-HDL was also able to significantly decrease LPS-induced leukocyte adhesion on endothelial cells and appeared to be more effective than normal HDL since lower concentrations were required. This inhibitory effect of HDL was not due to HDL itself but it requires preincubation of HDL with LPS. When HDL was separated into protein and lipid fractions, it was found that lipid-free apoHDL was able to significantly inhibit LPS-induced leukocyte adhesion, whereas lipid component of HDL had no effect. In conclusion, our studies suggested that HDL, both normal and acute-phase, could inhibit an inflammatory effect of LPS on endothelial cells in vivo. AP-HDL was more potent than normal HDL in inhibiting LPS-induced leukocyte adhesion, and this effect was attributed to the protein component of HDL.

Keywords: High-density lipoproteins (HDL), endotoxin, leukocyte adhesion, endothelial cells.

1. Introduction

High-density lipoprotein (HDL) is a group of lipoprotein particles which have the highest density in the circulation. HDL has several antiatherogenic effects, including the ability to transport excess cellular cholesterol to the liver for excretion, to protect low- density lipoprotein (LDL) against oxidation and to inhibit platelet aggregation [1].

Besides its pivotal role in protecting against atherosclerosis, accumulating evidence also suggests that HDL possesses anti-inflammatory effects and plays an important role in innate immunity. A number of *in vitro* and *in vivo* studies have demonstrated that HDL could bind endotoxin or lipopolysaccharide (LPS) of gram-negative bacteria resulting in detoxification of LPS [2-9]. *In vitro*, LPS bound to lipoprotein was 20- to 1,000-fold less active than the unbound form in inducing monocytes and macrophages to release cytokines [10]. *In vivo*, HDL also demonstrates anti-inflammatory properties. Intravenous infusion of reconstituted HDL or HDL apoprotein protected normal mice from the toxic effects of LPS [7]. When transgenic mice with 2-fold elevation of plasma HDL levels were injected with LPS, they had more LPS bound to HDL, lower plasma cytokine levels, and improved survival rates compared with control mice [7]. Beside the effects on monocytes and macrophages, LPS also activates endothelial cells. Infusion of reconstituted HDL inhibited infiltration of neutrophils and the expression of adhesion molecules on endothelial cells induced by a periarterial collar in rabbits [11]. However, whether HDL could inhibit the effects of LPS on endothelial cells *in vivo* has not been studied.

During bacterial infection, a wide range of alterations in metabolism occur. These are part of the body's reaction known as the acute-phase response (APR), which helps protect the host from further injury and facilitates the repair process [12]. The APR also induces a variety of alterations in lipid and lipoprotein metabolism [13]. HDL circulating during the APR, also called acute-

phase HDL (AP-HDL), has been shown to have different lipid and protein composition compared to that of normal HDL, which leads to alterations in various functions of HDL [13]. In this study, we determined whether normal and AP-HDL could inhibit LPS- induced leukocyte adhesion on endothelial cells *in vivo* and examined the component of HDL responsible for its effect.

2. Materials and Methods

2.1. Materials

Lipopolysaccharide (LPS, Escherichia coli 055:B5) was purchased from Sigma (USA). Centrifugal filter devices (molecular weight cutoff 10,000 Dalton) and 0.22 μm pore size filter units were from Millipore (Ireland). Quick-seal polyallomer tubes were from Beckman Coulter (USA). A modified Lowry assay kit was purchased from Pierce (USA). Various chemicals were purchased from Asia Pacific Specialty Chemicals (Australia), Merck (Germany), or Sigma (USA). Normal saline solution (NSS), and sterile water were from General Hospital Product Public (Thailand).

2.2. Isolation of normal HDL and acute-phase HDL (AP-HDL)

Male Syrian hamsters, 6 – 8 weeks of age, were purchased from the National Animal Center, Mahidol University (Thailand). They were maintained on standard laboratory chow and tap water ad libitum 5 – 7 days before the experiment. Syrian hamsters were used in these experiments because lipoprotein metabolism of hamsters closely resembles that of human than other rodents [14-16].

Hamsters were divided randomly into two groups ; one group received 100 μg of LPS/100 g body weight (BW) and the other received normal saline. Because LPS can cause anorexia, food was withdrawn after the injection in both groups. Sixteen hours after the injection, animals were anesthetized and blood samples were collected in a sterile fashion. Normal HDL and AP-HDL

were isolated by differential ultracentrifugation from pooled sera of hamsters injected with normal saline and LPS, respectively, [17]. Potassium bromide (KBr) was used to adjust for the desired density, and ultracentrifugation was performed using a Beckman ultracentrifuge. Normal HDL and AP-HDL were dialyzed against normal saline, filtered with sterile filters, and used within 2 weeks. Protein concentrations of HDL were determined by a modified Lowry method. Special precautions during isolation and handing of HDL were used to avoid the contamination [16].

2.3. Extraction of apoHDL and lipid of HDL.

Removal of lipids from HDL in the process of delipidation results in lipid-free protein component of HDL called apoHDL as briefly described below. Purified HDL was extracted with 10 volume of 3:1 (vol/vol) cold ethanol/diethyl ether and stored overnight at -20^{O}C. Then, the solution was centrifuged at -5^{O}C and the apoHDL protein pellet, and the lipid phase were separated. ApoHDL was washed with cold diethyl ether once. Both apoHDL and the lipid phase were dried under N_2 gas. ApoHDL was solubilized in phosphate buffer solution, filtered with a 0.22 μm sterile filter and its protein concentration was measured by a modified Lowry assay. Lipids were dissolved in 2:1 (vol/vol) chloroform-methanol [18].

2.4. Leukocyte adhesion on endothelial cells of the the mesentery.

Male Wistar rats (200-300 g) were obtained from the National Animal Center, Mahidol University (Thailand). They were maintained on standard laboratory chow and tap water ad libitum 5 – 7 days before the experiment.

After an overnight fast, rats were anesthetized with sodium pentobarbital (60 mg/kg BW i.p.). The carotid artery and the jugular vein were cannulated for measuring mean arterial blood pressure (MAP), and for agent administration, respectively. A midline laparotomy was made and a loop of mesentery was exteriorized and spreaded onto a Plexiglass chamber for microscopic

observation. The mesentery was fixed with a 37^0C–Krebs Ringer Solution (pH 7.4)–soaked gauze and superfused continuously with 37^0C Krebs Ringer Solution (pH 7.4) to avoid dehydration throughout the experiment.

The animal was then placed under a fluorescence microscope. A fluorescence videomicroscopic system (Nikon, Tokyo, Japan) were equipped with a videocamera (MTI SIT68), a videorecorder (Sony GUM-1411QM) and a videotimer (Sony, Japan). An objective lens (X20) was used, and video-images were recorded on videocassettes for off-line analysis.

After the rats were stabilized for 20-30 minutes, a 25-45 μm diameter postcapillary venule was chosen for observation. Acridine orange at the concentration of 1.8 mg/ml was i.v. injected as a bolus (0.25 ml) through the cannulated jugular vein. Twenty minutes after acridine orange injection, baseline quantification of leukocyte adhesion was recorded. Then, LPS was administered and leukocyte adhesion was recorded at time zero. In some studies, LPS was preincubated with normal HDL, AP-HDL, apoHDL, or lipids at 37^OC for 3 hours before use [19]. Leukocytes which remain stationary for 30 seconds on endothelial cells were counted at 1, 3, 5, 10, 15, 30, 45, 60, 75, and 90 minutes [20-21].

2.5. Data analysis

All data were presented as mean ± standard errors of the mean (mean ± SEM). Comparison among groups was performed by ANOVA and differences in pairs of means among groups were defined by Bonferroni test. The p-value of less than 0.05 indicates a significant difference between groups.

3. Results

3.1. Effects of LPS on leukocyte adhesion on endothelial cells of the mesentery.

LPS is known to induce leukocyte adhesion on endothelial cells. Figure 1 shows the dose response curve of LPS on leukocyte adhesion. Different concentrations of LPS (0.1 μg/100 g BW, 1 μg/100 g BW and 10 μg/100 g BW) could significantly induce leukocyte adhesion on endothelial cells of the mesentery. Therefore, the lowest dose of LPS (0.1 μg/100 g BW) was chosen for the next set of experiments.

3.2.Effects of normal HDL on LPS-induced leukocyte adhesion on endothelial cells of the mesentery.

Next, we examined whether normal HDL could inhibit LPS-induced leukocyte adhesion on endothelial cells. LPS was preincubated with normal HDL at $37^{O}C$ for 3 hours before administration. As shown in Figure 2, preincubation of LPS with normal HDL could significantly inhibit LPS-induced leukocyte adhesion on endothelial cells. We found that 10 μg of normal HDL/0.1 μg of LPS/100 g BW was required to completely inhibit LPS-induced leukocyte adhesion, whereas lower concentrations had no effect (Fig. 2).

3.3. Effects of AP-HDL on LPS-induced leukocyte adhesion on endothelial cells of the mesentery.

AP-HDL, which occurs during infection and inflammation, has different composition and function from normal HDL. We therefore tested whether AP-HDL could inhibit LPS-induced leukocyte adhesion in a similar fashion as we observed with normal HDL. The result showed that AP-HDL was able to inhibit LPS-induced leukocyte adhesion (Fig. 3), but lower concentrations of AP-HDL (5 μg of AP-HDL/0.1 μg of LPS/100 g BW) was required to completely inhibit LPS-induced leukocyte adhesion (Fig. 3). A comparison between different concentrations of normal HDL and AP-HDL that inhibit LPS-induced leukocyte adhesion is shown in Fig. 4.

3.4. Effects of HDL on LPS-induced leukocyte adhesion require incubation with LPS.

Since HDL itself might affect the leukocyte adhesion on endothelial cells, we therefore administered either normal HDL or AP-HDL without LPS into the rats. Fig. 5 shows that either normal HDL or AD-HDL alone did not have any effect in leukocyte adhesion on endothelial cells.

In addition, the inhibitory effect of HDL on LPS-induced leukocyte adhesion requires incubation with LPS. When HDL was immediately mixed with LPS without preincubation and administered, we found that HDL did not inhibit LPS-induced leukocyte adhesion as shown in Fig. 6.

3.5. Effects of normal apoHDL, AP apoHDL, lipid of normal HDL and lipid of AP-HDL on LPS-induced leukocyte adhesion on endothelial cells of the mesentery.

In order to investigate whether the effect of HDL on inhibiting LPS-induced leukocyte adhesion was due to lipid or protein component in HDL, we isolated lipid-free apoHDL, and lipids from HDL. Fig. 7 shows that after preincubation with LPS, both normal apoHDL and AP apoHDL could significantly inhibit LPS-induced leukocyte adhesion on endothelial cells. However, the lipid component of either normal HDL or AP-HDL did not have any effect on LPS-induced leukocyte adhesion on endothelial cells as shown in Fig. 8.

4. Discussion

Our study shows that both normal HDL and AP-HDL are able to inhibit LPS-induced leukocyte adhesion on endothelial cells *in vivo,* and that AP-HDL is more effective than normal HDL because lower concentrations of AP-HDL are required to completely inhibit LPS-induced leukocyte adhesion. This inhibitory effect of HDL is not a direct effect on endothelium but it requires

interaction between HDL and LPS. In addition, we identified that the protein, not lipid, component of HDL was responsible for this effect.

LPS is a membrane lipid of Gram-negative bacteria that acts as a potent inflammatory stimulus in humans and other mammals. *In vitro*, LPS can stimulate adhesion molecule expression of endothelial cells by stimulating cytokine and chemokine release from several cells types, including monocytes, macrophages, and endothelial cells [22-29]. *In vivo*, LPS is able to stimulate endothelial adhesion molecule expression [30] and leukocyte adhesion on endothelial cells [20, 31].

The inhibitory effects of HDL on cytokine-induced endothelial cell adhesion molecule expression have been demonstrated *in vitro* [32-36]. In addition, elevating HDL in the circulation leads to decreased cytokine-induced E-selectin expression by porcine microvascular endothelial cells [35]. Our current study further shows that normal HDL can inhibit LPS-induced leukocyte adhesion on endothelial cells *in vivo*. Moreover, AP-HDL is also able to inhibit LPS-induced leukocyte adhesion on endothelial cells *in vivo* and appears to be more effective than normal HDL.

However, both normal HDL and AP-HDL had no direct effect on leukocyte adhesion. It has previously been reported that HDL could not attenuate cellular adhesion molecules (CAMs) expression in arterial endothelium by itself [1].

Our study shows that this inhibitory effect of HDL requires preincubation of LPS with HDL which suggests the interaction between LPS and HDL. HDL preincubated with LPS that forms complex with LPS can render LPS less active in stimulating cytokine production from the macrophages [10]. Brandenburg K. et al. studied the interaction of HDL with LPS by a variety of physical techniques and biological assays and found that the functional groups of LPS interacting with HDL were the phosphates and the

diglucosamine backbone [37]. Our study suggests that it may be the protein component of HDL, not lipid, that interacts with LPS.

AP-HDL, which circulates during the APR, has different protein and lipid components from normal HDL, which leads to alterations of its function [13]. This study demonstrates that AP-HDL is more effective than normal HDL in inhibiting LPS-induced leukocyte adhesion on endothelial cells. Although it is known that there are changes in many HDL-associated proteins during the APR, at this point, we cannot determine which protein(s) of HDL exhibits this inhibitory effect. One of the protein candidates is lipopolysaccharide binding protein (LBP) [13]. LBP is one of the HDL-associated protein which can bind LPS and its level increases during the APR. Normally, LBP can bind and transfer LPS not only to the receptor on the surface of macrophages and monocytes but also to lipoproteins [38]. *In vitro*, addition of low concentrations of LBP to macrophages enhanced LPS-induced TNF-α synthesis, but acute-phase concentrations of LBP were found to block this effect. In addition, high levels of LBP inhibited LPS-mediated cytokine release and reduced mortality rate *in vivo* [39]. High levels of LBP during the APR may increase LPS transfer into AP-HDL, protecting against the toxic effect of LPS.

In conclusion, both normal HDL and AP-HDL can inhibit LPS-induced leukocyte adhesion on endothelial cells but AP-HDL appears to be more effective. Investigations into the active protein components of HDL that interact with LPS and inhibit its effect may provide further insights and leads to new protein target(s) to ameliorate the toxic effect of LPS.

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References

1. A. K. Stannard, S. Khan, A. Graham, J. S. Owen, S. P. Allen, Inability of plasma high-density lipoproteins to inhibit cell adhesion mocule expression in human coronary artery endothelial cells, *Atherosclerosis.* **154** (2001), 31-8.

2. D. B. Weinstein, R. J. Ulevitch, A new protective role for high density lipoprotein (HDL): detoxification of endotoxin, *Circulation.* **58(Suppl. II)** (1979), II-90 (Abstr.).

3. R. J. Ulevitch, A. R. Johnston, D. B. Weinstein, New function for high density lipoproteins : their participation in intravascular reactions of bacterial lipopolysaccharides, *J. Clin. Invest.* **64** (1979), 1516-24.

4. R. J. Ulevitch, A. R. Johnston, D. B. Weinstein, New function for high density lipoproteins : isolation and characterization of a bacterial lipopolysaccharide-high density lipoprotein complex formed in rabbit plasma, *J. Clin. Invest.* **67** (1981), 827-37.

5. R. S. Munford, C. L. Hall, J. M. Dietschy, Binding of Salmonella typhimurium lipopolysaccharides to rat high-density lipoproteins, *Infect. Immun.* **34** (1981), 835-43.

6. K. Emancipator, G. Csako, R. J. Elin, *In vitro* inactivation of bacterial endotoxin by human lipoproteins and apolipoproteins, *Infect. Immun.* **60** (1992), 596-601.

7. D. M. Levine, T. S. Parker, T. M. Donnelly, A. Walsh, A. L. Rubin, *In vivo* protection against endotoxin by plasma high-density lipoprotein, *Proc Natl. Acad. Sci. U.S.A.* **90** (1993), 12040-4.

8. T. S. Parker, D. M. Levine, J. C. C. Chang, J. Baxter, C. C. Coffin, A. L. Rubin, Reconstituted high-density lipoprotein neutralizes gram-negative bacterial lipopolysaccharides in human whole blood, *Infect. Immun.* **63** (1995), 253-8.

9. J. H. M. Levels, P. R. Abraham, A. van den Ende, S. J. H. van Deventer, Distribution and kinetics of lipoprotein-bound endotoxin, *Infect. Immun.* **69** (2001), 2821-8.

10. J. M. Cavaillon, C. Fitting, N. Haeffner-Cavaillon, S. J. Kirsch, H. S. Warren, Cytokine response by monocytes and macrophages to free and lipoprotein-bound lipopolysaccharide, *Infect. Immun.* **58(7)** (1990), 2375-82.

11. S. J. Nicholls, G. J. Dusting, B. Cutri, S. Bao, G. R. Drummond, K.-A. Rye and et al., Reconstituted high-density lipoproteins inhibit the acute pro-oxidant and proinflammatory vascular changes induced by a periarterial collar in normocholesterolemic rabbits, *Circulation.* **111** (2005), 1543-50.

12. C. Gabay, I. Kushner, Acute-phase proteins and other systemic responses to inflammation, *N. Engl. J. Med.* **340** (1999), 448-54.

13. W. Khovidhunkit, M. S. Kim, R. A. Memon, J. K. Shigenaga, A. H. Moser, K. R. Feingold, C. Grunfeld, Effects of infection and inflammation on lipid and lipoprotein metabolism : mechanisms and consequences to the host, *J. Lipid Res.* **45** (2004), 1169-96.

14. H. Ly, L. F. Omar, J. F. Christopher, K. S. Judy, H. M. Arther, G. Carl, et al., Endotoxin and TNF lead to reduced plasma LCAT activity and decreased hepatic LCAT mRNA levels in syrian hamsters, *J. Lipid Res.* **36** (1995), 1254-63.

15. L. A. Woollett, D. K. Spady, Kinetic parameters for high density lipoprotein apolipoprotein A-I and cholesteryl ester transport in the hamster, *J. Clin. Invest.* **99** (1997), 1704-13.

16. W. Khovidhunkit, J. K. Shigenaga, A. H. Moser, K. R. Feingold, C. Grunfeld, Cholesterol efflux by acute-phase high density lipoprotein: role of lecithin: cholesterol acyltransferase, *J. Lipid Res.* **42** (2001), 967-75.

17. R. J. Havel, H. A. Eder, J. H. Bragdon, The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum, *J. Clin. Invest.* **34(9)** (1955), 1345-53.

18. W. M. Sperry, F. C. Brand, The determination of total lipids in blood serum, *J. Biol. Chem.* **213** (1955), 69-76.

19. C. Grunfeld, M. Marshall, J. K. Shigenaga, A. H. Moser, P. Tobias, K. R. Feingold, Lipoproteins inhibit macrophage activation by lipoteichoic acid, *J. Lipid Res.* **40** (1999), 245-52.

20. K. L. Davenpeck, J. Zagorski, R. P. Schleimer, B. S. Bochner, Lipopolysaccharide-induced leukocyte rolling and adhesion in the rat mesenteric microcirculation : regulation by glucocorticoids and role of cytokines, *J. Immunol.* **161** (1998), 6861-70.

21. D. Chakraphan, P. Sridulyakul, B. Thipakorn, S. Bunnag, V. H. Huxley, S. Patumraj, Attenuation of endothelial dysfunction by exercise training in STZ-induced diabetic rats, *Clin. Hemorheol. Microcirc.* **32(3)** (2005), 217-26.

22. A. Mantovani, E. Dejana, Cytokines as communication signals between leukocytes and endothelial cells, *Immunol. Today.* **10(11)** (1989), 370-5.

23. J. Pugin, C. C. Schurer-Maly, D. Leturcq, A. Moriarty, R. J. Ulevitch, P. S. Tobias, Lipopolysaccharide activation of human endothelial and epithelial cells is mediated by lipopolysaccharide-binding protein and soluble CD14, *Proc. Natl. Acad. Sci. USA.* **90** (1993), 2744-8.

24. A. Haziot, G. W. Rong, J. Silver, S. M. Goyert, Recombinant soluble CD14 mediates the activation of endothelial cells by lipopolysaccharide, *J. Immunol.* **151(3)** (1993), 1500-7.

25. J. Schletter, H. Heine, A. J. U lmer, E. T. Rietscel, Molecular mechanisms of endotoxin activity, *Arch. Microbiol.* **164** (1995), 383-9.

26. Q. Jiang, S. Akashi, K. Miyake, H. R. Petty, Cutting edge : lipopolysaccharide induces physical proximity between CD14 and toll-like receptor 4 (TLR4) prior to nuclear translocation of NF-κB, *J. Immunol*, **165** (2000), 3541-4.

27. J. C. Chow, D. W. Young, D. T. Golenbock, W. J. Christ, F. Gusovsky, Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction, *J. Biol. Chem.* **274(16)** (1999), 10689-92.

28. J. Pugin, R. J. Ulevitch, P. S. Tobias, A critical role for monocytes and CD14 in endotoxin-induced endothelial cell activation, *J. Exp. Med.* **178** (1993), 2193-200.

29. M. A. Dentener, V. Bazil, E. J. U. Von Asmuth, M. Ceska, W. A. Buurman, Involvement of CD14 in lipopolysaccharide-induced tumor necrosis factor-α, IL-6 and IL-8 release by human monocytes and alveolar macrophages, *J. Immunol.* **150** (1993), 2885-91.

30. B. G. Yipp, G. Andonegui, C. J. Howlett, S. M. Robbins, T. Hartung, M. Ho, et al., Profound differences in leukocyte-endothelial cell responses to lipopolysaccharide versus lipoteichoic acid, *J. Immunol.* **168** (2002), 4650-8.

31. R. C. Woodman, D. Teoh, D. Payne, P. Kubes, Thrombin and leukocyte recruitment in endotoxemia, *Am. J. Physiol. Heart. Circ. Physiol.* **279** (2000), 1338-45.

32. D. T. Ashby, K.-A. Rye, M. A. Clay, M. A. Vadas, J. R. Gamble, P. J. Barter, Factors influencing the ability of HDL to inhibit expression of vascular cell adhesion molecule-1 in endothelial cells, *Arterioscler Thromb Vasc Biol.* **18** (1998), 1450-5.

33. D. Ashby, J. Gamble, M. Vadas, N. Fidge, S. Siggins, K.-A. Rye., et al., Lack of effect of serum amyloid A (SAA) on the ability of high-density

lipoproteins to inhibit endothelial cell adhesion molecule expression, *Atherosclerosis.* **154** (2001), 113-21.

34. G. W. Cockerill, K.-A. Rye, J. R. Gamble, M. A. Vadas, P. J. Barter, High-density lipoproteins inhibit cytokine-induced expression of endothelial cell adhesion molecules, *Arterioscler Thromb Vasc Biol.* **15** (1995), 1987-1994.

35. G. W. Cockerill, T. Y. Huehns, A. Weerasinghe, C. Stocker, P. G. Lerch, N. E. Miller, et al., Elevation of plasma high-density lipoprotein concentration reduces interleukin-1-induced expression of E-selectin in an in vivo model of acute inflammation, *Circulation.* **103** (2001), 108-112.

36. P. W. Baker, K.-A. Rye, J. R. Gamble, M. A. Vadas, P. J. Barter, Ability of reconstituted high density lipoproteins to inhibit cytokine-induced expression of vascular cell adhesion molecule-1 in human umbilical vein endothelial cells, *J. Lipid Res.* **40** (1999), 345-53.

37. K. Brandenburg, G. Jurgens, J. Andra, B. Lindner, M. H. J. Koch, A. Blume, et al., Biophysical characterization of the interaction of high - density lipoprotein (HDL) with endotoxins, *Eur. J. Biochem.* **269** (2002), 5972-81.

38. M. M. Wurfel, S. T. Kunitake, H. Lichenstein, J. P. Kane, S. D. Wright, Lipopolysaccharide (LPS)-binding protein is carried on lipoproteins and acts as a cofactor in the neutralization of LPS, *J. Exp. Med.* **180** (1994), 1025-35.

39. N. Lamping, R. Dettmer, N. W. J. Schroder, D. Pfeil, W. Hallatschek, R. Burger, et al., LPS-binding protein protects mice from septic shock caused by LPS or gram-negative bacteria, *J. Clin. Invest.* **101(10)** (1998), 2065-71.

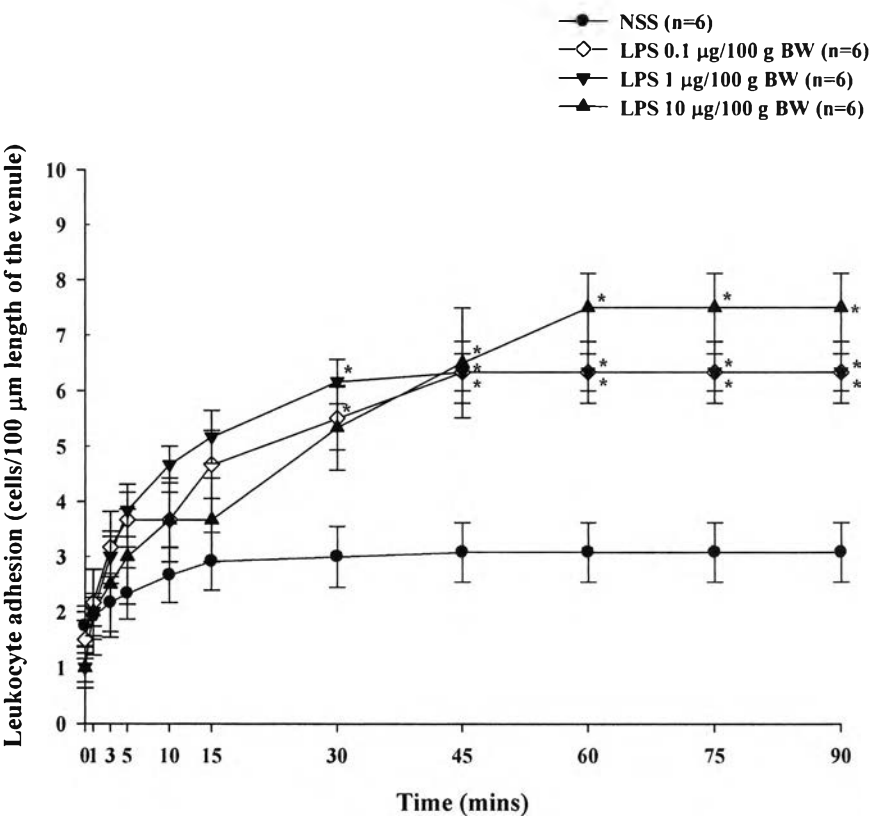

Fig. 1. Effects of various concentrations of LPS on leukocyte adhesion on endothelial cells. For NSS-treated group, NSS was preincubated at 37^{O}C for 3 hours. LPS was preincubated with NSS for LPS-treated group at 37^{O}C for 3 hours. After i.v. administration, leukocyte adhesion on endothelial cells of the mesenteric venules was counted as described in materials and methods. * P<0.05, VS. NSS group.

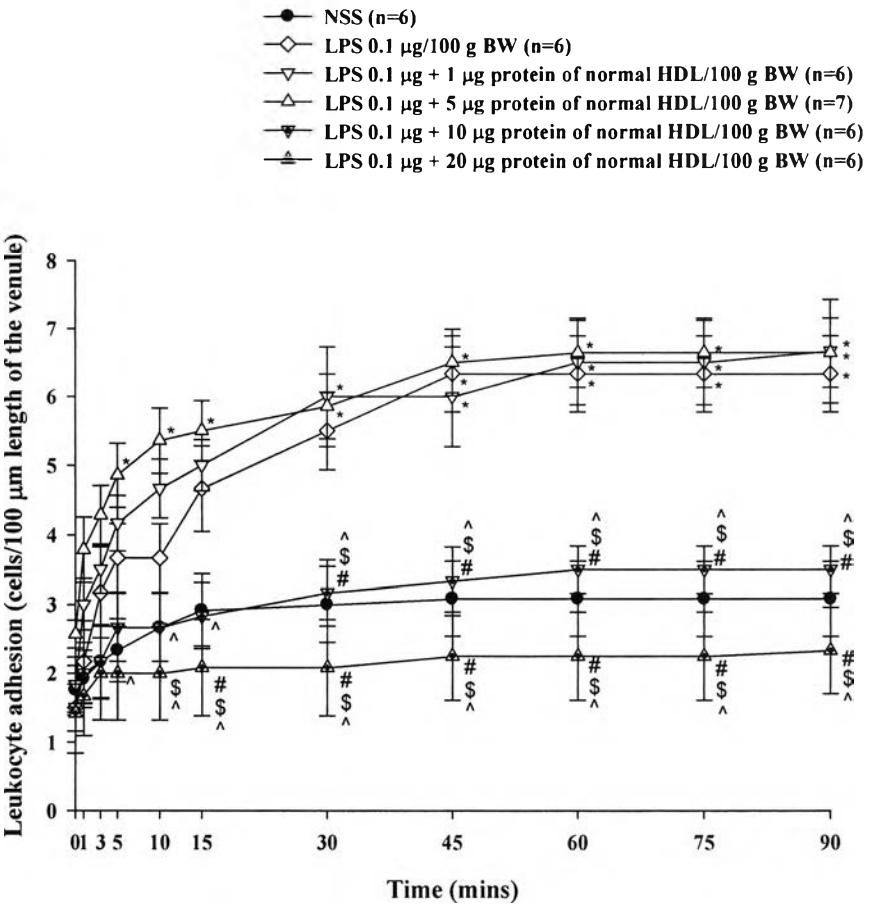

Fig. 2. Effects of normal HDL on LPS-induced leukocyte adhesion on endothelial cells. For NSS-treated group, NSS was preincubated at 37°C for 3 hours. LPS was preincubated with NSS for LPS-treated group or different concentrations of normal HDL for LPS+normal HDL-treated group at 37°C for 3 hours. After i.v. administration, leukocyte adhesion on endothelial cells of the mesenteric venules was counted as described in materials and methods. *P<0.05, VS. NSS group; #P<0.05, VS. LPS group; $P<0.05, VS. LPS+1 μg protein of normal HDL group; ^P<0.05, VS. LPS+5 μg protein of normal HDL group.

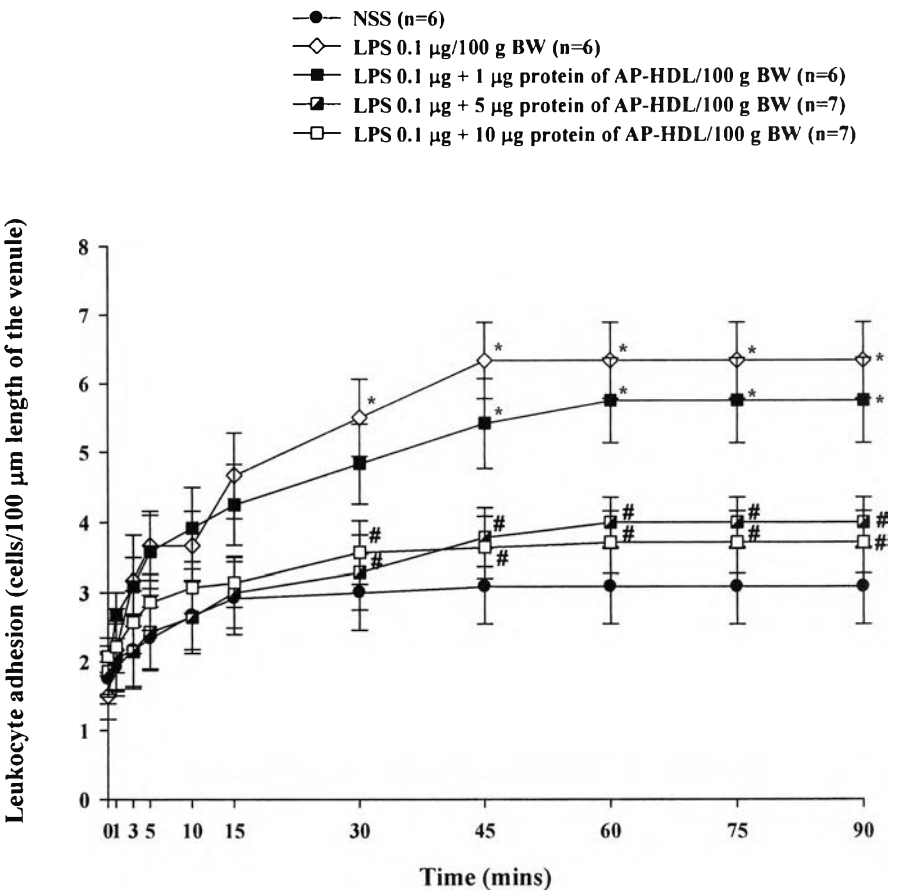

Fig. 3. Effects of AP-HDL on LPS-induced leukocyte adhesion on endothelial cells. For NSS-treated group, NSS was preincubated at 37°C for 3 hours. LPS was preincubated with NSS for LPS-treated group or different concentrations of AP-HDL for LPS+AP-HDL-treated group at 37°C for 3 hours. After i.v. administration, leukocyte adhesion on endothelial cells of the mesenteric venules was counted as described in materials and methods. *P<0.05, VS. NSS group; #P<0.05, VS. LPS group.

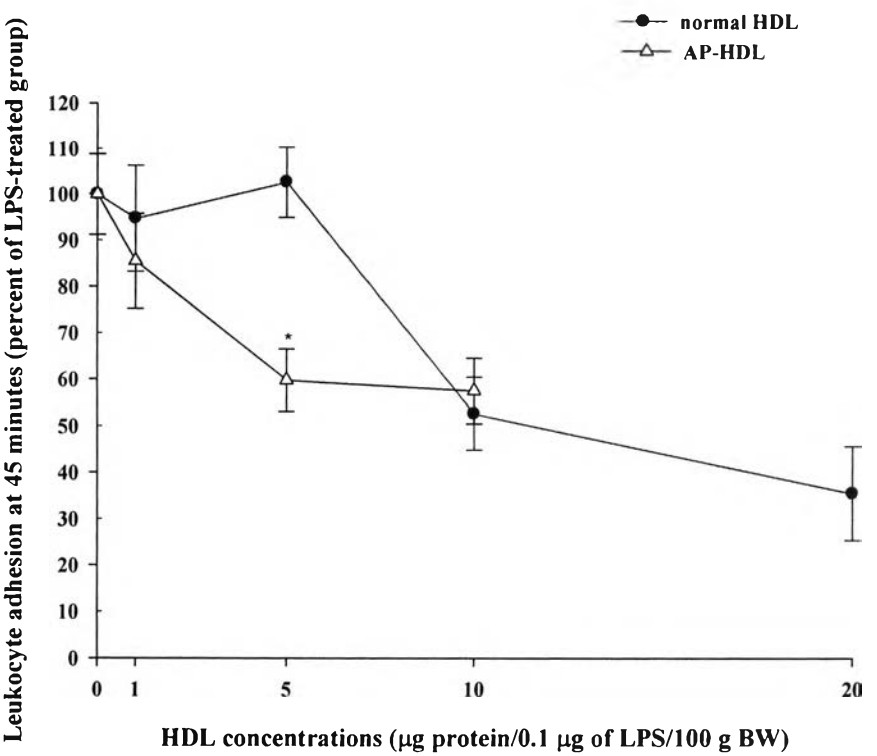

Fig. 4. Effects of various concentrations of HDL on LPS-induced leukocyte adhesion on endothelial cells. LPS was preincubated with NSS or normal HDL or AP-HDL with increasing concentrations of HDL as indicated in the abscissa at 37°C for 3 hours. After i.v. administration, leukocyte adhesion on endothelial cells of the mesenteric venules at 45 minutes was counted as described in materials and methods. Numbers of leukocyte adhesion were presented as percent of LPS-treated group. They were calculated from (y/z)100. y represented numbers of leukocyte adhesion of LPS+normal HDL or LPS+AP-HDL group. Z represented numbers of leukocyte adhesion of LPS-treated group. *P<0.05, VS. normal HDL group.

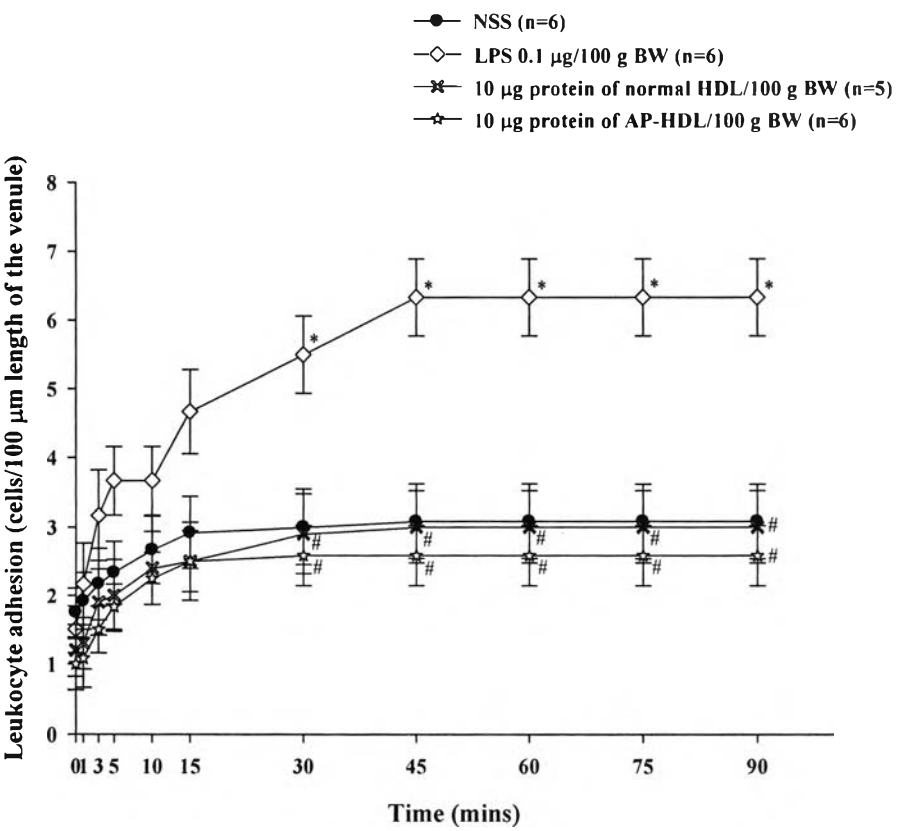

Fig. 5. Effects of LPS and HDL on LPS-induced leukocyte adhesion on endothelial cells. NSS, LPS or HDL was preincubated at 37^{0}C for 3 hours. After i.v. administration, leukocyte adhesion on endothelial cells of the mesenteric venules was counted as described in materials and methods. *P<0.05, VS. NSS group; $^{\#}$P<0.05, VS. LPS group.

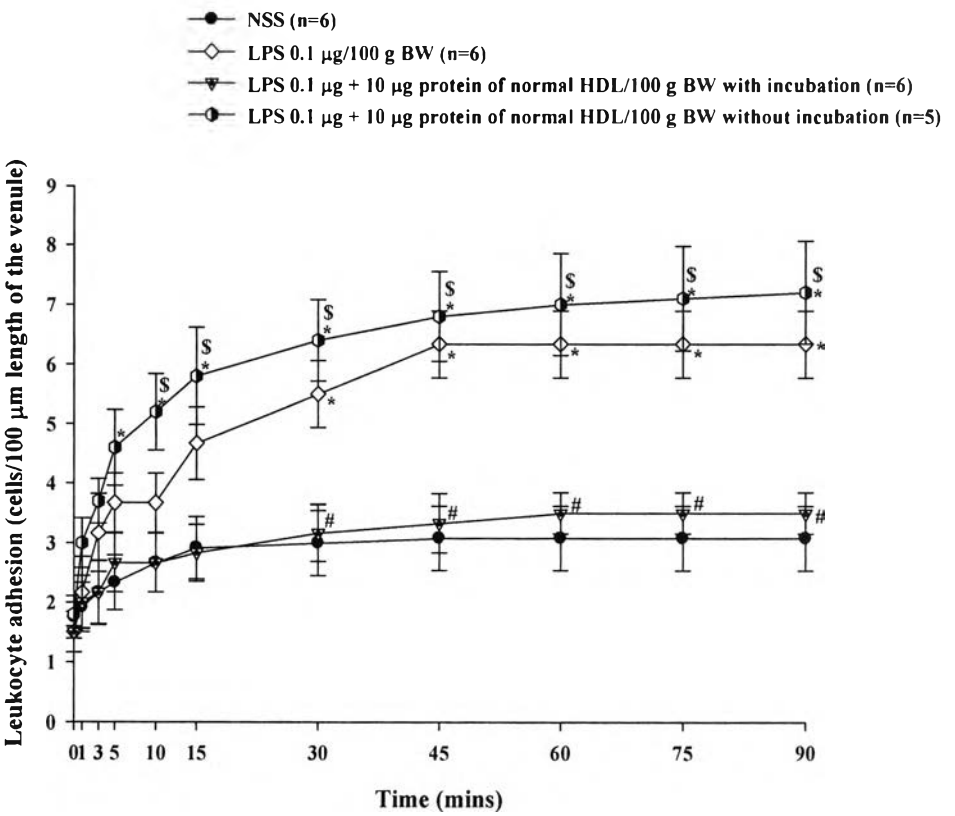

Fig. 6. Effects of incubation between LPS and normal HDL on LPS-induced leukocyte adhesion on endothelial cells. NSS was preincubated for NSS-treated group and LPS was preincubated with NSS for LPS-treated group at 37^{O}C for 3 hours. LPS was or was not preincubated with normal HDL at 37^{O}C for 3 hours. After i.v. administration, leukocyte adhesion on endothelial cells of the mesenteric venules was counted as described in materials and methods. *P<0.05, VS. NSS group; $^{\#}$P<0.05, VS. LPS group; $^{\$}$P<0.05, VS. LPS+normal HDL with incubation group.

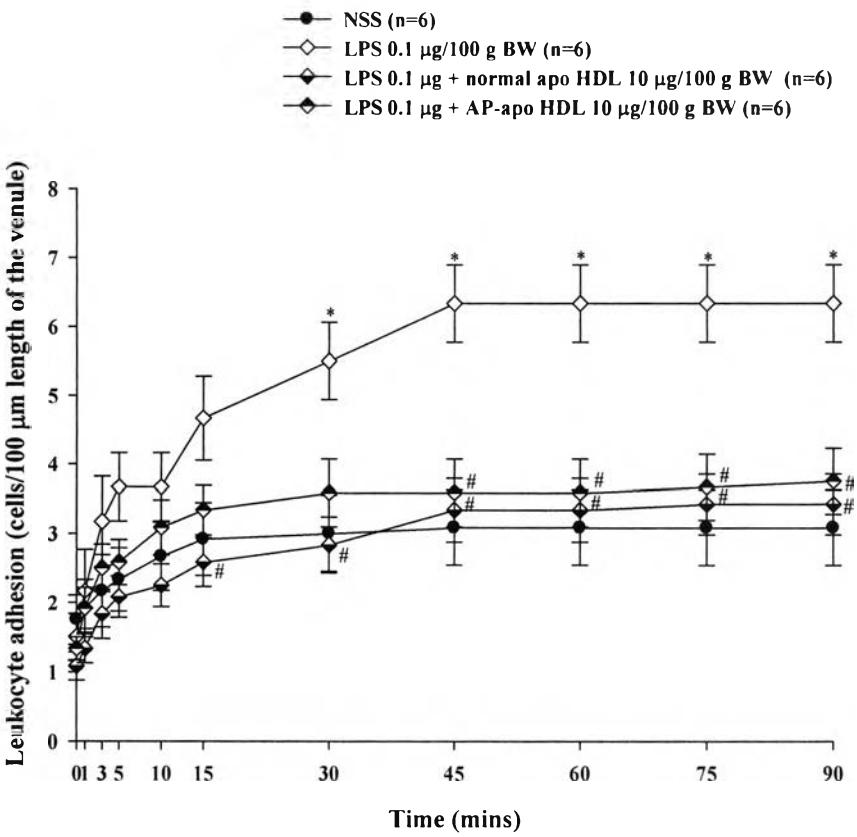

Fig. 7. Effects of apoHDL on LPS-induced leukocyte adhesion on endothelial cells. For NSS-treated group, NSS was preincubated at 37°C for 3 hours. LPS was preincubated with NSS for LPS-treated group or apoHDL for LPS+apoHDL-treated group at 37°C for 3 hours. After i.v. administration, leukocyte adhesion on endothelial cells of the mesenteric venules was counted as described in materials and methods. *P<0.05, VS. NSS group; #P<0.05, VS. LPS group.

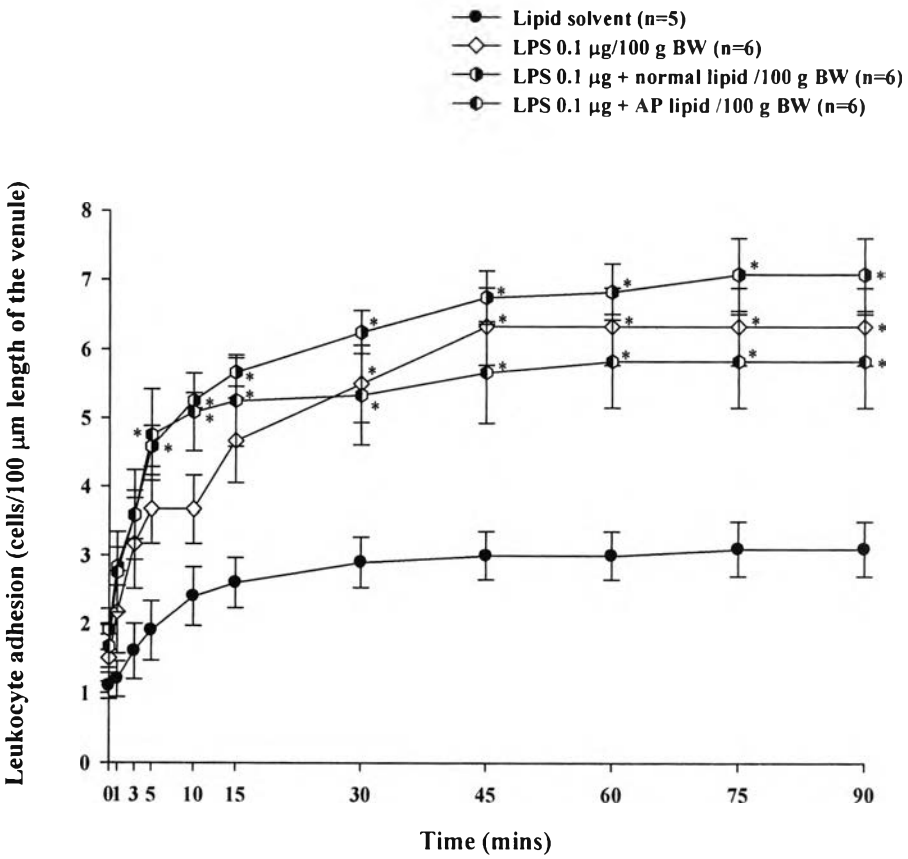

Fig. 8. Effects of lipids of HDL on LPS-induced leukocyte adhesion on endothelial cells. For lipid solvent-treated group, lipid solvent was preincubated with NSS at 37^{O}C for 3 hours. LPS was preincubated with NSS for LPS-treated group or lipids of HDL for LPS+lipids of HDL-treated group at 37^{O}C for 3 hours. After i.v. administration, leukocyte adhesion on endothelial cells of the mesenteric venules was counted as described in materials and methods. *P<0.05, VS. lipid solvent group.

APPENDIX II

RAW DATA

Table 6 Effects of HDL on the growth of *E. coli.*

Incubation time	Percentage of control (no HDL)					
	No HDL		Normal HDL		AP-HDL	
	MEAN	SEM	MEAN	SEM	MEAN	SEM
0	100.00	0.00	100.00	0.0000	100.00	0.00
0.5	100.00	0.00	136.93	25.37	191.92	63.70
1	100.00	0.00	111.23	14.59	183.89	61.27
2	100.00	0.00	124.32	26.10	245.90	75.29
4	100.00	0.00	179.83	30.89	123.27	23.99
6	100.00	0.00	260.59	64.50	227.24	97.18
24	100.00	0.00	145.71	27.53	127.48	13.06

Table 7 Effects of various concentrations of HDL on the growth of *E. coli* at 6 hours.

Concentrations of HDL	Percentage of control (no HDL)					
	No HDL		Normal HDL		AP-HDL	
	MEAN	SEM	MEAN	SEM	MEAN	SEM
50	100.00	0.00	180.49	83.80	225.84	123.26
100	100.00	0.00	98.09	14.84	237.40	65.52
200	100.00	0.00	260.59	64.49	227.24	97.18
400	100.00	0.00	378.33	274.48	286.76	133.58
800	100.00	0.00	241.13	112.09	325.95	182.83
1670	100.00	0.00	176.41	81.44	131.03	48.78

Table 8 Effects of HDL on the growth of *S. epidermidis*.

Incubation time	Percentage of control (no HDL)					
	No HDL		Normal HDL		AP-HDL	
	MEAN	SEM	MEAN	SEM	MEAN	SEM
0	100.00	0.00	100.00	0.00	100.00	0.00
0.5	100.00	0.00	112.47	4.71	103.85	3.82
1	100.00	0.00	116.20	5.67	122.00	13.58
2	100.00	0.00	102.05	18.53	264.59	74.08
4	100.00	0.00	160.78	68.15	595.92	256.43
6	100.00	0.00	1290.38	1126.52	1835.54	1048.83
24	100.00	0.00	84.64	6.56	119.67	9.65

Table 9 Effects of various concentrations of HDL on the growth of *S. epidermidis* at 6 hours.

Concentrations of HDL	Percentage of control (no HDL)					
	No HDL		Normal HDL		AP-HDL	
	MEAN	SEM	MEAN	SEM	MEAN	SEM
50	100.00	0.00	102.04	40.17	909.42	806.76
100	100.00	0.00	87.95	40.51	213.45	105.04
200	100.00	0.00	319.09	288.53	907.14	751.38
400	100.00	0.00	447.09	242.99	10452.62	8881.46
800	100.00	0.00	25659.47	11744.49	69471.19	36588.37
1670	100.00	0.00	4431.39	2418.99	35034.60	18592.14

Table 10 Effects of NSS on leukocyte adhesion on endothelial cells.

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	2	3	4	4	4	4	4	4	4	4	4
2	2.5	2.5	2.5	2.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
3	0	0	0	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
4	2	2	2	2.5	2.5	.5	4	4	4	4	4
5	2	2	2	2	3	3.5	3.5	3.5	3.5	3.5	3.5
6	2	2	2.5	2.5	2.5	2.5	2.5	3	3	3	3
MEAN	1.75	1.92	2.17	2.33	2.67	2.92	3.00	3.08	3.08	3.08	3.08
SEM	0.36	0.42	0.53	0.46	0.49	0.52	0.55	0.54	0.54	0.54	0.54

Table 11 Effects of 10 μg/100 g BW of LPS on leukocyte adhesion on endothelial cells.

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	2	5	6	6	6	6	7	7	7	7	7
2	2	2	3	4	5	5	5	8	8	8	8
3	1	3	4	4	4	4	7	7	9	9	9
4	0	0	0	0	1	1	5	9	9	9	9
5	0	0	0	2	2	2	2	2	5	5	5
6	1	2	2	2	4	4	6	6	7	7	7
MEAN	1.00	2.00	2.50	3.00	3.67	3.67	5.33	6.50	7.50	7.50	7.50
SEM	0.37	0.77	0.96	0.86	0.76	0.76	0.76	0.99	0.62	0.62	0.62

Table 12 Effects of 1 μg/100 g BW of LPS on leukocyte adhesion on endothelial cells.

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	1	2	3	3	4	4	6	6	6	6	6
2	0	2	2	3	4	5	5	6	6	6	6
3	1	2	3	4	5	5	7	7	7	7	7
4	1	1	2	3	5	6	7	7	7	7	7
5	2	3	4	6	6	7	7	7	7	7	7
6	1	2	4	4	4	4	5	5	5	5	5
MEAN	1.00	2.00	3.00	3.83	4.67	5.17	6.17	6.33	6.33	6.33	6.33
SEM	0.26	0.26	0.37	0.48	0.33	0.48	0.40	0.33	0.33	0.33	0.33

Table 13 Effects of 0.1 μg/100 g BW of LPS on leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	1	1	3	3	3	4	6	8	8	8	8
2	2	2	2	3	3	3	4	6	6	6	6
3	2	3	5	5	5	6	7	7	7	7	7
4	2	3	3	4	4	4	5	6	6	6	6
5	0	0	1	2	2	4	4	4	4	4	4
6	2	4	5	5	5	7	7	7	7	7	7
MEAN	1.50	2.17	3.17	3.67	3.67	4.67	5.50	6.33	6.33	6.33	6.33
SEM	0.34	0.60	0.65	0.49	0.49	0.61	0.56	0.56	0.56	0.56	0.56

Table 14 Effects of 20 μg protein of normal HDL on 0.1 μg /100 g BW of LPS-induced leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	3	3	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
2	0	0	0.5	0.5	0.5	0.5	0.5	1.5	1.5	1.5	1.5
3	3	3	3	3	3	3	3	3	3	3	3
4	0.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	2
5	0	0	0	0	0	0	0	0	0	0	0
6	2	2.5	2.5	2.5	2.5	3	3	3	3	3	3
MEAN	1.42	1.67	2.00	2.00	2.00	2.08	2.08	2.25	2.25	2.25	2.33
SEM	0.58	0.57	0.68	0.68	0.68	0.70	0.70	0.64	0.64	0.64	0.63

Table 15 Effects of 10 μg protein of normal HDL on 0.1 μg /100 g BW of LPS-induced leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	2	3	4	4	4	4	4	4	4	4	4
2	0	0	0	1	1	1	1	1	2	2	2
3	1	2	2	2	2	2	3	3	3	3	3
4	2	3	3	4	4	4	4	4	4	4	4
5	2	2	2	2	2	3	4	4	4	4	4
6	2	2	2	3	3	3	3	4	4	4	4
MEAN	1.50	2.00	2.17	2.67	2.67	2.83	3.17	3.33	3.50	3.50	3.50
SEM	0.34	0.45	0.54	0.49	0.49	0.48	0.48	0.49	0.34	0.34	0.34

Table 16 Effects of 5 μg protein of normal HDL on 0.1 μg /100 g BW of LPS-induced leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	3	4.5	4.5	6	7	7	7.5	8	8	8	8
2	2	2	3	3	3.5	3.5	3.5	4	4	4	4
3	3	4	5	5.5	5.5	5.5	6.5	6.5	7	7	7
4	2	4.5	5	5.5	6.5	6.5	6.5	7.5	7.5	7.5	7.5
5	3	5	5.5	6	6	6	6	7	7.5	7.5	7.5
6	3	4.5	4.5	4.5	4.5	5	5.5	6.5	6.5	6.5	6.5
7	2	2	2.5	3.5	4.5	5	5.5	6	6	6	6
MEAN	2.57	3.79	4.29	4.86	5.36	5.50	5.86	6.50	6.64	6.64	6.64
SEM	0.20	0.47	0.42	0.46	0.47	0.44	0.47	0.49	0.51	0.51	0.51

Table 17 Effects of 1 μg protein of normal HDL on 0.1 μg /100 g BW of LPS-induced leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	2	4	4	5	5	5	5	5	5	5	5
2	2	3	4	4	5	6	9	9	9	9	10
3	3	3	4	5	6	6	6	6	7	7	7
4	2	2	2	3	3	4	5	5	5	5	5
5	0	2	3	3	4	4	4	4	6	6	6
6	2	4	4	5	5	5	7	7	7	7	7
MEAN	1.83	3.00	3.50	4.17	4.67	5.00	6.00	6.00	6.50	6.50	6.67
SEM	0.40	0.37	0.34	0.40	0.42	0.37	0.73	0.73	0.62	0.62	0.76

Table 18 Effects of 10 μg protein of AP-HDL on 0.1 μg /100 g BW of LPS-induced leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	3	3	4.5	4.5	4.5	4.5	6	6	6	6	6
2	2	2	2	2.5	2.5	3	3	3.5	4	4	4
3	1.5	1.5	1.5	1.5	2	2	2.5	2.5	2.5	2.5	2.5
4	1	1	1.5	2	2	2	2.5	2.5	2.5	2.5	2.5
5	3	4	4	4	4	4	4	4	4	4	4
6	2	2	2.5	2.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
7	2	2	2	3	3	3	3.5	3.5	3.5	3.5	3.5
MEAN	2.07	2.21	2.57	2.86	3.07	3.14	3.57	3.64	3.71	3.71	3.71
SEM	0.28	0.38	0.46	0.40	0.37	0.36	0.46	0.45	0.45	0.45	0.45

Table 19 Effects of 5 μg protein of AP-HDL on 0.1 μg /100 g BW of LPS-induced leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	2	2.5	2.5	2.5	3	3	3	3	3	3	3
2	3	3	3	3	3	3	3.5	4.5	4.5	4.5	4.5
3	1	1	1	1	1	1	1	2.5	4	4	4
4	1	1	1	1.5	1.5	2	2.5	3	3	3	3
5	3	4	4.5	5	5	5	5	5	5	5	5
6	1	1	1	2	2.5	3.5	3.5	3.5	3.5	3.5	3.5
7	2	2	2	2	2.5	3.5	4.5	5	5	5	5
MEAN	1.86	2.07	2.14	2.43	2.64	3.00	3.29	3.79	4.00	4.00	4.00
SEM	0.37	0.48	0.54	0.53	0.52	0.51	0.54	0.42	0.35	0.35	0.35

Table 20 Effects of 1 μg protein of AP-HDL on 0.1 μg /100 g BW of LPS-induced leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	2	2.5	3.5	3.5	4	5	6	6.5	7	7	7
2	1	1.5	1.5	2	2	2	2.5	3	3.5	3.5	3.5
3	1	3	3	3.5	3.5	4	4.5	4.5	5	5	5
4	3	3.5	4.5	5.5	6	6	6.5	7.5	7.5	7.5	7.5
5	2	2	2.5	2.5	3	3.5	4.5	6	6.5	6.5	6.5
6	2	3.5	3.5	4.5	5	5	5	5	5	5	5
MEAN	1.83	2.67	3.08	3.58	3.92	4.25	4.83	5.42	5.75	5.75	5.75
SEM	0.31	0.33	0.42	0.52	0.58	0.57	0.57	0.65	0.62	0.62	0.62

Table 21 Effects of 10 μg protein of normal HDL/100 g BW on leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	1	1.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
2	2	2	2.5	3	4	4	4	4	4	4	4
3	1	1	1	1	1.5	1.5	1.5	2	2	2	2
4	0	0	0.5	0.5	1	1	2	2	2	2	2
5	2	2	3	3	3	3.5	4.5	4.5	4.5	4.5	4.5
MEAN	1.2	1.3	1.9	2	2.4	2.5	2.9	3	3	3	3
SEM	0.37	0.37	0.48	0.52	0.53	0.57	0.58	0.52	0.52	0.52	0.52

Table 22 Effects of 10 μg protein of AP-HDL/100 g BW on leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	2	2	2.5	3	4	4.5	4.5	4.5	4.5	4.5	4.5
2	1	1	1.5	2	2	2	2	2	2	2	2
3	2	2.5	2.5	2.5	2.5	3	3	3	3	3	3
4	0	0	0.5	1	1.5	2	2.5	2.5	2.5	2.5	2.5
5	0	0	1	1	2	2	2	2	2	2	2
6	1	1	1	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
MEAN	1.00	1.08	1.50	1.83	2.25	2.50	2.58	2.58	2.58	2.58	2.58
SEM	0.37	0.42	0.34	0.33	0.38	0.45	0.44	0.44	0.44	0.44	0.44

Table 23 Effects of incubation between 0.1 μg/100 g BW of LPS and 10 μg protein of AP-HDL/100 g BW on leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	2	4	4.5	6.5	7.5	8.5	9	9.5	10	10	10
2	1	2	2.5	2.5	3.5	3.5	5	5	5	5	5
3	2	2.5	3.5	5	5	5	6	6.5	6.5	6.5	7
4	2	2.5	3.5	4.5	5	6	6	6	6	6	6
5	2	4	4.5	4.5	5	6	6	7	7.5	8	8
MEAN	1.8	3	3.7	4.6	5.2	5.8	6.4	6.8	7	7.1	7.2
SEM	0.20	0.42	0.37	0.64	0.64	0.82	0.68	0.75	0.85	0.87	0.86

Table 24 Effects of 10 μg normal apoHDL on 0.1 μg/100 g BW of LPS induced leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 mir
1	1	1.5	2.5	3	3	3	3.5	4	4	4	4
2	0.5	1	1	1.5	2	2	2.5	2.5	2.5	2.5	2.5
3	1.5	1.5	2	2	2	2	2	2.5	2.5	3	3
4	0.5	0.5	0.5	1	1	1.5	1.5	2	2	2	2
5	1.5	1.5	2.5	2.5	2.5	3.5	4	4.5	4.5	4.5	4.5
6	1.5	2	2.5	2.5	3	3.5	3.5	4.5	4.5	4.5	4.5
MEAN	1.08	1.33	1.83	2.08	2.25	2.58	2.83	3.33	3.33	3.42	3.42
SEM	0.20	0.21	0.36	0.30	0.31	0.35	0.40	0.46	0.46	0.44	0.44

Table 25 Effects of 10 μg AP-apoHDL on 0.1 μg/100 g BW of LPS-induced leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	1.5	2	2	2	2.5	3.5	3.5	3.5	3.5	3.5	3.5
2	1.5	2.5	3	3	3	3	3	3	3	3.5	4
3	1	1.5	2	2	2.5	3	3	3	3	3	3
4	2	3	4	4	5	5	6	6	6	6	6
5	1	1	2	2	2.5	2.5	3	3	3	3	3
6	1	1.5	2	2.5	3	3	3	3	3	3	3
MEAN	1.33	1.92	2.50	2.58	3.08	3.33	3.58	3.58	3.58	3.67	3.75
SEM	0.17	0.30	0.34	0.33	0.40	0.36	0.49	0.49	0.49	0.48	0.48

Table 26 Effects of lipids of normal HDL on 0.1 μg/100 g BW of LPS-induced leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	2	3	3.5	4.5	5	5.5	6	6.5	6.5	6.5	6.5
2	3	3.5	4.5	5.5	5.5	6.5	7	8	8	8.5	8.5
3	2.5	3.5	4	4	5	5	5	5.5	5.5	5.5	5.5
4	1.5	2	4	5	5.5	5.5	6	6	6	6	6
5	1.5	3	3.5	5	5	6	6.5	7.5	7.5	7.5	7.5
6	1	2	2	3.5	5.5	5.5	7	7	7.5	8.5	8.5
MEAN	1.92	2.83	3.58	4.58	5.25	5.67	6.25	6.75	6.83	7.08	7.08
SEM	0.30	0.28	0.35	0.30	0.11	0.21	0.31	0.38	0.40	0.52	0.52

Table 27 Effects of lipids of AP-HDL on 0.1 μg/100 g BW of LPS-induced leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	1.5	3	3.5	4	4	4	4	4	4	4	4
2	2.5	4.5	5	6.5	6.5	7.5	8	8.5	8.5	8.5	8.5
3	0.5	0.5	1	2.5	4	4	4	4	5	5	5
4	2.5	4	4.5	6	6	6	6	6.5	6.5	6.5	6.5
5	1.5	2	2.5	3.5	3.5	3.5	3.5	4.5	4.5	4.5	4.5
6	1.5	2.5	5	6	6.5	6.5	6.5	6.5	6.5	6.5	6.5
MEAN	1.67	2.75	3.58	4.75	5.08	5.25	5.33	5.67	5.83	5.83	5.83
SEM	0.31	0.59	0.65	0.67	0.57	0.67	0.73	0.74	0.68	0.68	0.68

Table 28 Effects of lipid solvent on leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	1	1	1.5	2	2	2	2.5	2.5	2.5	2.5	2.5
2	1.5	2	2.5	3	4	4	4	4	4	4	4
3	1	1	1	1.5	2	2.5	2.5	3	3	3	3
4	0.5	0.5	0.5	0.5	1.5	2	2	2	2	2	2
5	1.5	1.5	2.5	2.5	2.5	2.5	3.5	3.5	3.5	4	4
MEAN	1.10	1.20	1.60	1.90	2.40	2.60	2.90	3.00	3.00	3.10	3.10
SEM	0.19	0.25	0.40	0.43	0.43	0.37	0.37	0.35	0.35	0.40	0.40

Table 29 Effects of 10 μg apo A-I on 0.1 μg/100 g BW of LPS-induced leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	2	2	2	2.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
2	1	1	1	1	1.5	2	2	2	2.5	2.5	2.5
3	2	2	2	2	2	2	2	2	2	2	2
4	1	1	1	1	1	1	1	1	1.5	1.5	1.5
5	2	2	2	2	3	3	4	4	4	4	4
6	1	1	1	1	1.5	1.5	2.5	2.5	2.5	2.5	2.5
MEAN	1.50	1.50	1.50	1.58	2.08	2.17	2.50	2.50	2.67	2.67	2.67
SEM	0.22	0.22	0.22	0.27	0.40	0.38	0.45	0.45	0.38	0.38	0.38

Table 30 Effects of 5 μg apo A-I on 0.1 μg/100 g BW of LPS-induced leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	2	2	2	2	2.5	3	4	4	4.5	4.5	4.5
2	2	3	3	3	3	3.5	4	4	4	4	4
3	3	3.5	4	4	4	4	4	4	4	4	4
4	2	2	2	2	2	2	2.5	3.5	3.5	3.5	3.5
5	1	1	2	2.5	2.5	3	3	3.5	4	4	4
MEAN	2.00	2.30	2.60	2.70	2.80	3.10	3.50	3.80	4.00	4.00	4.00
SEM	0.32	0.44	0.40	0.37	0.34	0.33	0.32	0.12	0.16	0.16	0.16

Table 31 Effects of 2.5 μg apo A-I on 0.1 μg/100 g BW of LPS-induced leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 μm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	2	2	2	2	2	3	3.5	3.5	4	4	5
2	2	3.5	4.5	5.5	5.5	6.5	6.5	6.5	6.5	6.5	8
3	1	1.5	1.5	2	3	3.5	4	4	4.5	4.5	4.5
4	2	4.5	5.5	5.5	5.5	5.5	5.5	6	6	6	6
5	1	3.5	4	4.5	4.5	4.5	5	5	5	5	5
6	2	3	4	5	5	5	5.5	6	6	6	6
MEAN	1.67	3.00	3.58	4.08	4.25	4.67	5.00	5.17	5.33	5.33	5.75
SEM	0.21	0.45	0.62	0.68	0.59	0.53	0.45	0.49	0.40	0.40	0.51

Table 32 Effects of 5 µg /100 g BW of apo A-I on leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 µm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	2	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
2	1.5	1.5	2	2	2	2.5	2.5	2.5	2.5	2.5	2.5
3	1.5	2	2	2.5	3	3.5	4	4.5	4.5	4.5	4.5
4	1	1.5	2	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
5	3	3	4	4.5	4.5	5	5	5	5	5	5
6	2	3	3.5	4	4	4	4	4	4	4	4
MEAN	1.83	2.25	2.67	3.00	3.08	3.33	3.42	3.50	3.50	3.50	3.50
SEM	0.28	0.28	0.36	0.41	0.40	0.42	0.44	0.47	0.47	0.47	0.47

Table 33 Effects of incubation between 0.1 µg/100 g BW of LPS and 5 µg/100 g BW of apo A-I on leukocyte adhesion on endothelial cells

Rat No	Leukocyte adhesion (cells/100 µm)										
	o min	1 min	3 min	5 min	10 min	15 min	30 min	45 min	60 min	75 min	90 min
1	3	3	3	4	5	5	5	5.5	6	6	6.5
2	2.5	3.5	4.5	5.5	5.5	6	7.5	8.5	8.5	9	9
3	2	4	5.5	5.5	6	6.5	6.5	6.5	6.5	6.5	6.5
4	2	2.5	3.5	3.5	3.5	4	4.5	4.5	4.5	4.5	4.5
5	3	4.5	6	6	6.5	7	8	8	8.5	8.5	8.5
6	2	3	4.5	6	6.5	6.5	7	7	7	7	7
MEAN	2.42	3.42	4.50	5.08	5.50	5.83	6.42	6.67	6.83	6.92	7.00
SEM	0.20	0.30	0.47	0.44	0.47	0.46	0.57	0.61	0.63	0.68	0.66

BIOGRAPHY

NAME	Miss Premtip Thaveeratitham
DATE OF BIRTH	December, 15, 1966
PLACE OF BIRTH	Bangkok, Thailand
EDUCATION	Chiangmai University, 1986 – 1989 : Bachelor of Science (Physical Therapy, First Hons.)
	Mahidol University, 1990 - 1992 : Master of Science (Physiology)
	Chulalongkorn University, 2001 – 2005 : Ph.D. candidate (Physiology)
POSITIONS	1992 – 1995 : Lecturer, Faculty of Associated Medical Sciences, Chiangmai University
	1995 – present : Lecturer, Faculty of Allied Health Sciences, Chulalongkorn University
PUBLICATIONS	Krishnamra, N. and Taweerathitam, P. Acute effect of prolactin on active calcium absorption in rats. Can. J. Physiol. Pharmacol. 73(8) (1995): 1185 – 9.
	Thaveeratitham, P., Khovidhunkit, W., Patumraj, S. High – density lipoproteins (HDL) inhibit endotoxin-induced leukocyte adhesion on endothelium in rats : effect of the acute-phase HDL. Clin. Hemorheol. Microcirc. (submitted).
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