CHAPTER IV

RESULTS

This part is composed of three sections which corresponds to the experimental protocol as described previously :

1. The inhibitory effects of normal HDL and AP-HDL on the growth of gram-negative and gram-positive bacteria

2. The effects of normal HDL and AP-HDL on LPS-induced leukocyte adhesion on endothelium.

3. The effects of the molecular components (apoHDL, lipids and apo A-I) of normal HDL and AP-HDL on LPS-induced leukocyte adhesion on endothelium

1. The inhibitory effects of normal HDL and AP-HDL on the growth of gram-negative or gram-positive bacteria

1.1. Effects of normal HDL and AP-HDL on the growth of gram-negative bacteria.

HDL has been demonstrated to inhibit the growth of bacteria *in vitro* (Tada et al., 1993). To determine whether normal HDL and AP-HDL could suppress the growth of gram-negative bacteria, *E. coli*, purified normal HDL and AP-HDL at the concentration of 200 μg/ml protein of HDL were incubated with *E. coli* and the bacterial growth as percent of the no HDL group was determined at different time points (0, 0.5, 1, 2, 4, 6, and 24 hours). It was found that both normal HDL and AP-HDL were not able to significantly inhibit growth of *E. coli* after incubation up to 24 hours as shown in figure 20.

Various concentrations of normal HDL and AP-HDL, up to physiological concentration in serum (50, 100, 200, 400, 800, and 1,670 μg/ml protein of HDL), did not have significant effect on the growth of *E. coli* (Figure 21).

1.2. Effects of normal HDL and AP-HDL on the growth of gram-positive bacteria.

Next, the effects of normal HDL and AP-HDL on the growth of gram-positive bacteria, *S. epidermidis*, were examined. Purified normal HDL and AP-HDL at the concentration of 200 μg/ml protein of HDL were incubated with *S. epidermidis* and the bacterial growth as percent of the no HDL group was determined at different time points (0, 0.5, 1, 2, 4, 6, 24 hours). Both normal HDL and AP-HDL could not significantly inhibit the growth of *S. epidermidis* up to 24 hours of incubation (Figure 22).

Similarly, when the concentrations of normal HDL and AP-HDL were varied from 50, 100, 200, 400, 800 to 1,670 μg/ml protein of HDL, all concentrations of normal HDL and AP-HDL were not able to significantly inhibit growth of *S. epidermidis* as shown in figure 23.

2. The effects of normal HDL and AP-HDL on LPS-induced leukocyte adhesion on endothelium

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

LPS is known to induce leukocyte adhesion on endothelial cells. Figure 24 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.

2.2. 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 25, 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 (Figure 25).

2.3. 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 (Figure 26), 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 (Figure 26). A comparison between different concentrations of normal HDL and AP-HDL that inhibit LPS-induced leukocyte adhesion is shown in figure 27.

2.4. 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. Figure 28 showed 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 figure 29.

3. The effects of the molecular components (apoHDL, lipids and apo A-I) of normal HDL and AP-HDL on LPS-induced leukocyte adhesion on endothelium.

3.1. Effects of normal apoHDL, AP apoHDL, lipids of normal HDL and lipids 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 protein or lipid component of HDL. Therefore, we isolated lipid-free apoHDL, and lipids from HDL. Figure 30 showed that after preincubation with LPS, both 10 μg/ml of normal apoHDL and 10 μg/ml of 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 figure 31. Because lipid solvent might affect leukocyte adhesion on endothelial cells, it therefore was administered into the rats after incubation with normal saline solution for 3 hours. The results found that lipid solvent did not significantly affect leukocyte adhesion on endothelial cells (Figure 31).

3.2. Effects of normal human apo A-I on LPS-induced leukocyte adhesion on endothelial cells.

Apo A-I is the main protein in HDL. In order to investigate whether the effect of apoHDL on inhibiting LPS-induced leukocyte adhesion was due to lipid-free apo A-I, lipid-free apo A-I was purified from normal human HDL.

Figure 32 showed that apo A-I at the concentrations of 5 μg/0.1 μg of LPS/100 g BW and 10 μg/0.1 μg of LPS/100 g BW of purified human apo A-I was able to significantly inhibit effects of LPS on leukocyte adhesion on endothelial cells.

3.3. Effects of normal human apo A-I on LPS-induced leukocyte adhesion on endothelial cells require incubation with LPS.

Because human apo A-I itself might affect the leukocyte adhesion on endothelial cells, purified apo A-I without LPS therefore was administered into the rats. Figure 33 showed that human apo A-I alone did not have any effect on leukocyte adhesion on endothelial cells.

In addition, the inhibitory effect of normal human apo A-I on leukocyte adhesion on endothelial cells required 3 hours incubation with LPS. When purified apo A-I was immediately mixed with LPS without preincubation and administered, purified apo A-I was not able to significantly inhibit LPS-induced leukocyte adhesion on endothelial cells as shown in figure 34.

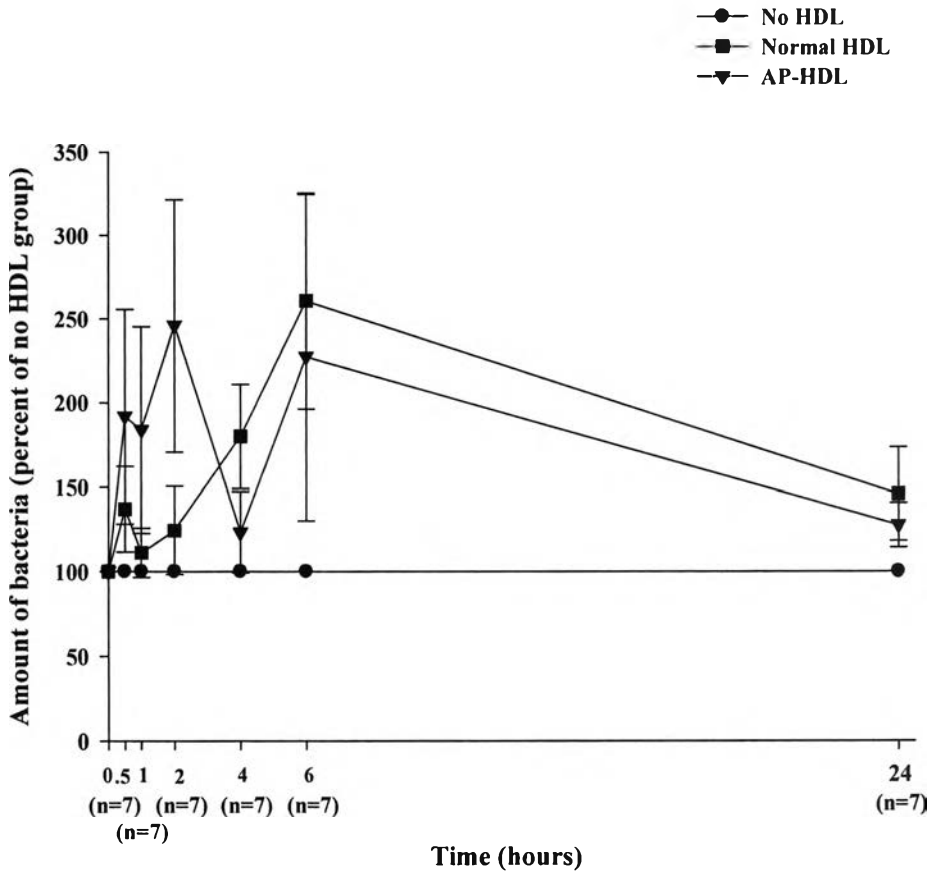

Figure 20 **Effects of HDL on the growth of *E. coli*. *E. coli* was incubated with HDL at 37^{O}C and sample at each time points was collected to determine the colonies of bacteria as described in materials and methods.**

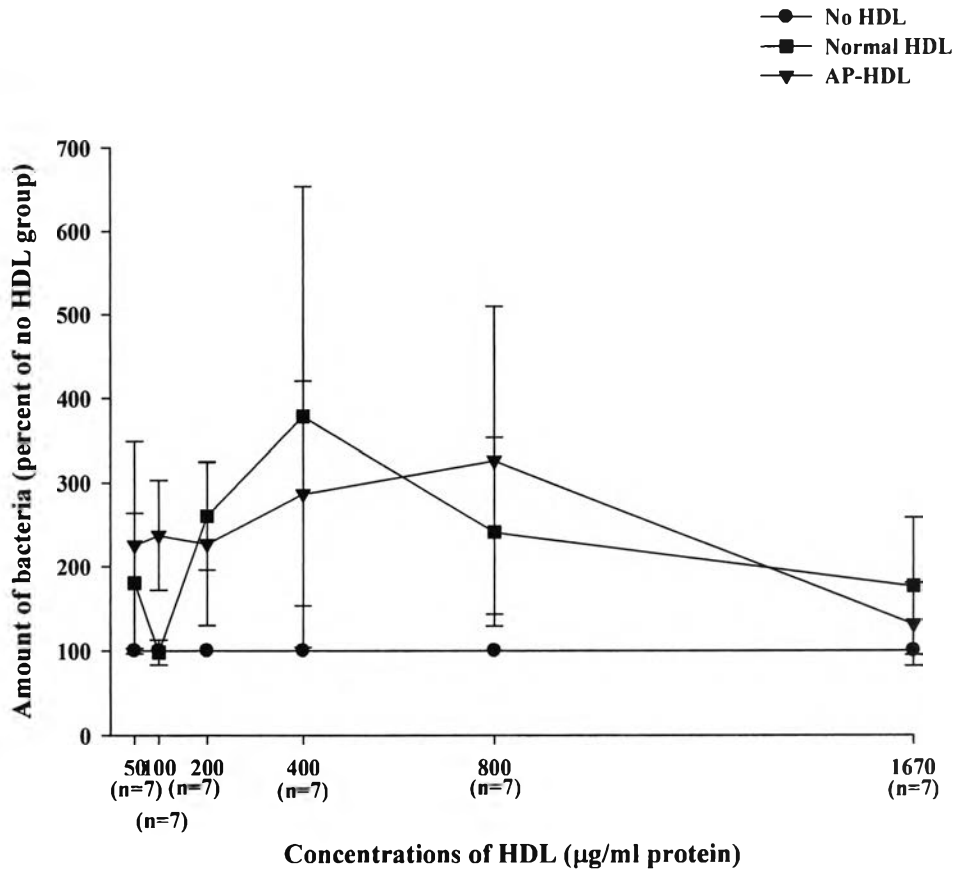

Figure 21 **Effects of various concentrations of HDL on the growth of *E. coli*. *E. coli* was incubated with HDL at 37^{O}C for 6 hours and sample was collected at each time points to determine the colonies of bacteria as described in materials and methods.**

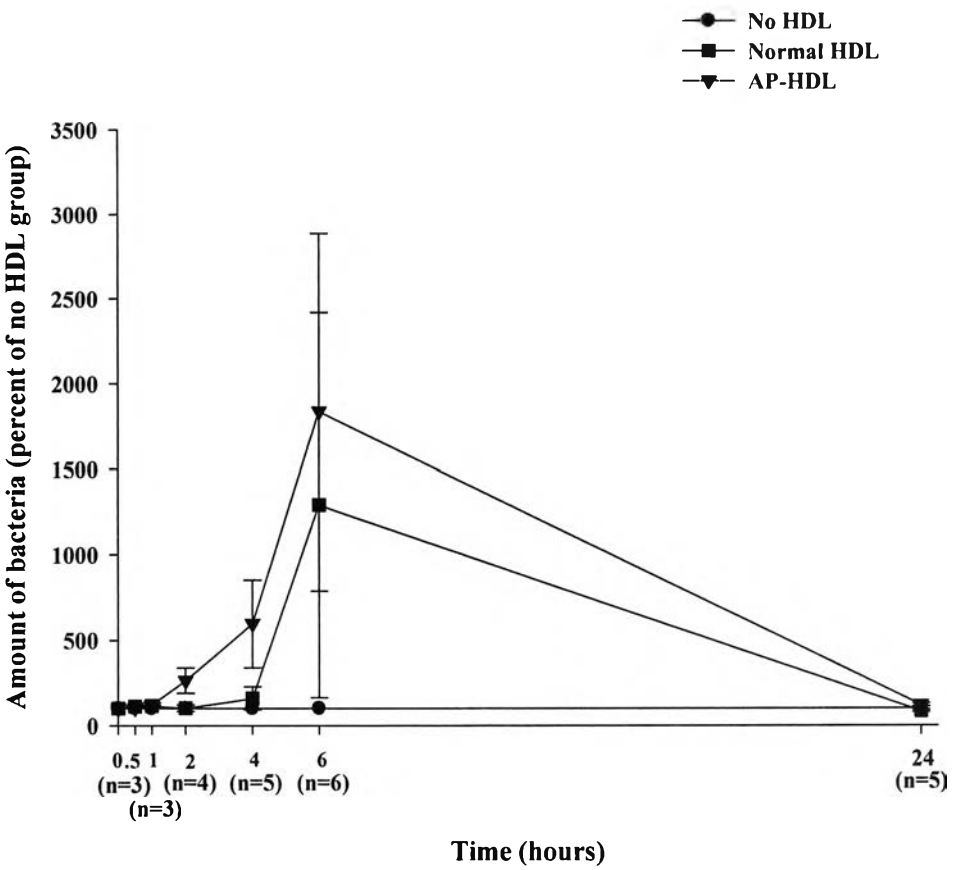

Figure 22 **Effects of HDL on the growth of *S. epidermidis*. *S. epidermidis* was incubated with HDL at 37^{O}C and sample at each time points was collected to determine the colonies of bacteria as described in materials and methods.**

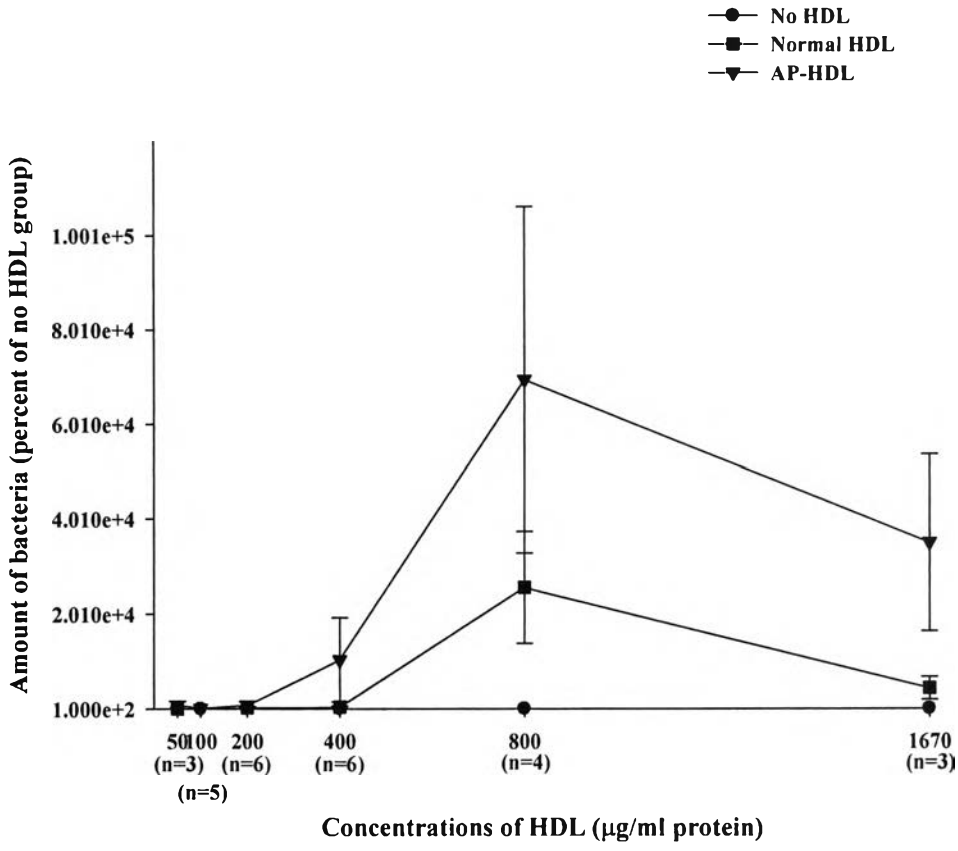

Figure 23 **Effects of various concentrations of HDL on the growth of *S. epidermidis*. *S. epidermidis* was incubated with HDL at 37^{O}C for 6 hours and sample at each time points was collected to determine the colonies of bacteria as described in materials and methods.**

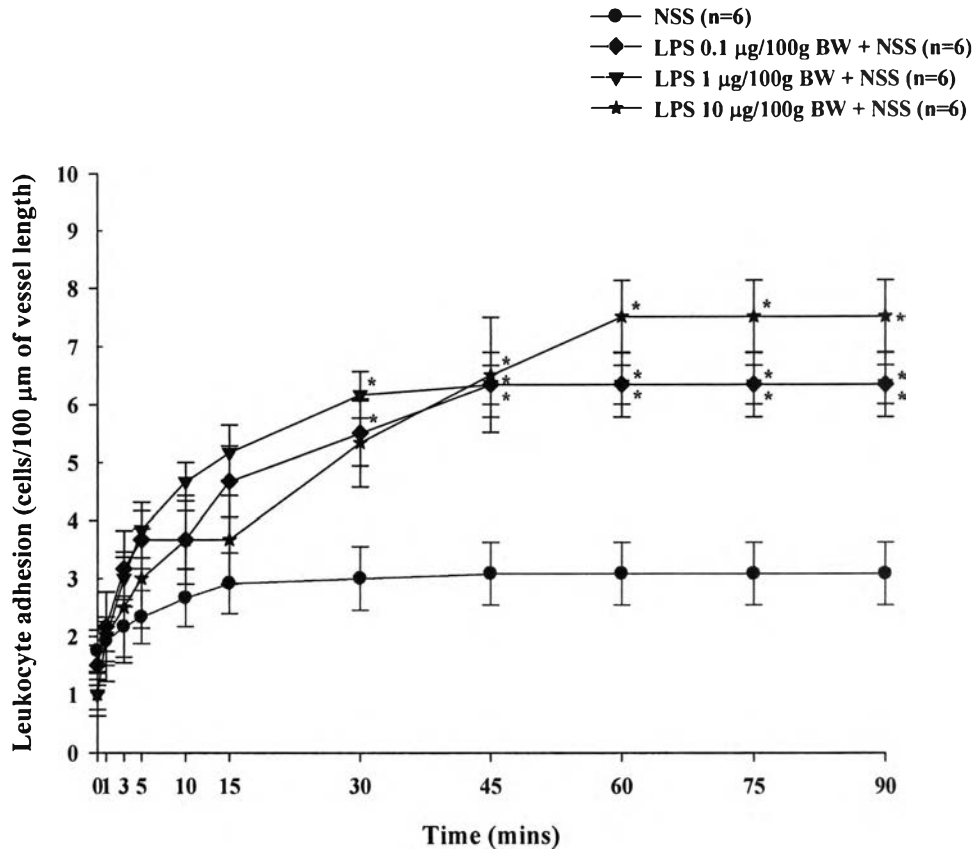

Figure 24 **Effects of various concentrations of LPS on leukocyte adhesion on endothelial cells. For NSS-treated group (—●—), NSS was preincubated at 37°C for 3 hours. LPS was preincubated with NSS for LPS-treated group (—◆—, —▼—, —★—) at 37°C for 3 hours. After i.v. administration, leukocyte adhesion on endothelial cells of the mesenteric venules was counted as described in materials and methods.**

*** P<0.05 : significant difference from NSS group.**

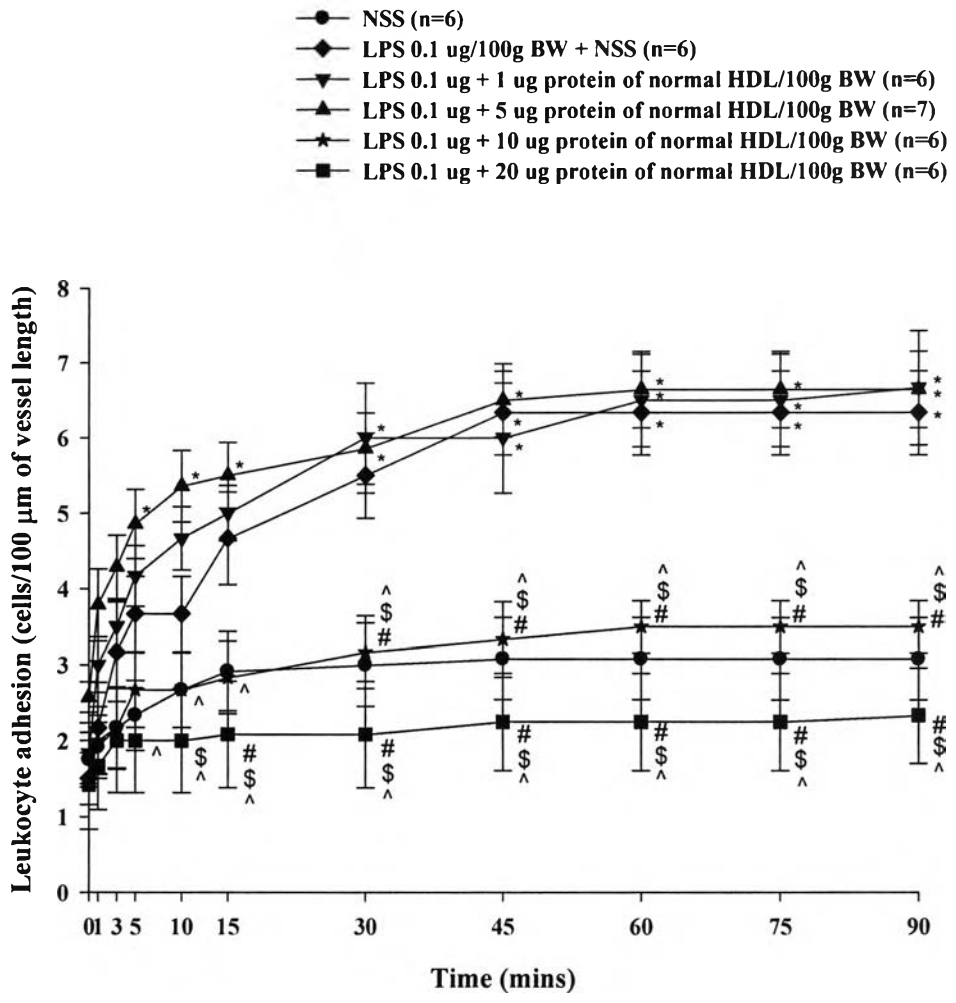

Figure 25 **Effects of normal HDL on LPS-induced leukocyte adhesion on endothelial cells. For NSS-treated group (—●—), NSS was preincubated at 37^{O}C for 3 hours. LPS was preincubated with NSS for LPS-treated group (—◆—) or different concentrations of normal HDL for LPS+normal HDL-treated group (—▼—, —▲—, —★—, —■—) 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 : significant difference from NSS group.
$^{\#}$P<0.05 : significant difference from LPS group.
$^{\$}$P<0.05 : significant difference from LPS+1 µg protein of normal HDL group.
$^{\wedge}$P<0.05 : significant difference from LPS+5 µg protein of normal HDL group.

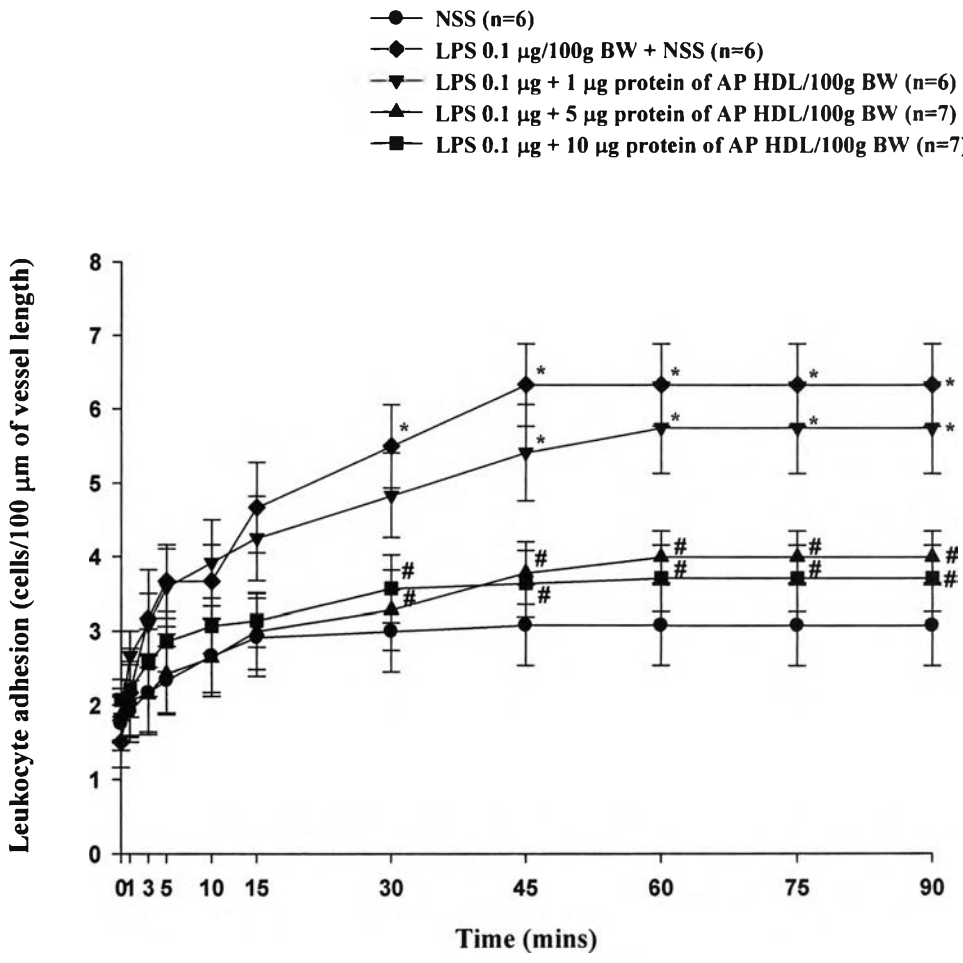

Figure 26 **Effects of AP-HDL on LPS-induced leukocyte adhesion on endothelial cells. For NSS-treated group (—●—), NSS was preincubated at 37^{O}C for 3 hours. LPS was preincubated with NSS for LPS-treated group (—◆—) or different concentrations of AP-HDL for LPS+AP-HDL-treated group (—▼—,—▲—,—■—) at 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 : significant difference from NSS group.
$^{\#}$P<0.05 : significant difference from LPS group.

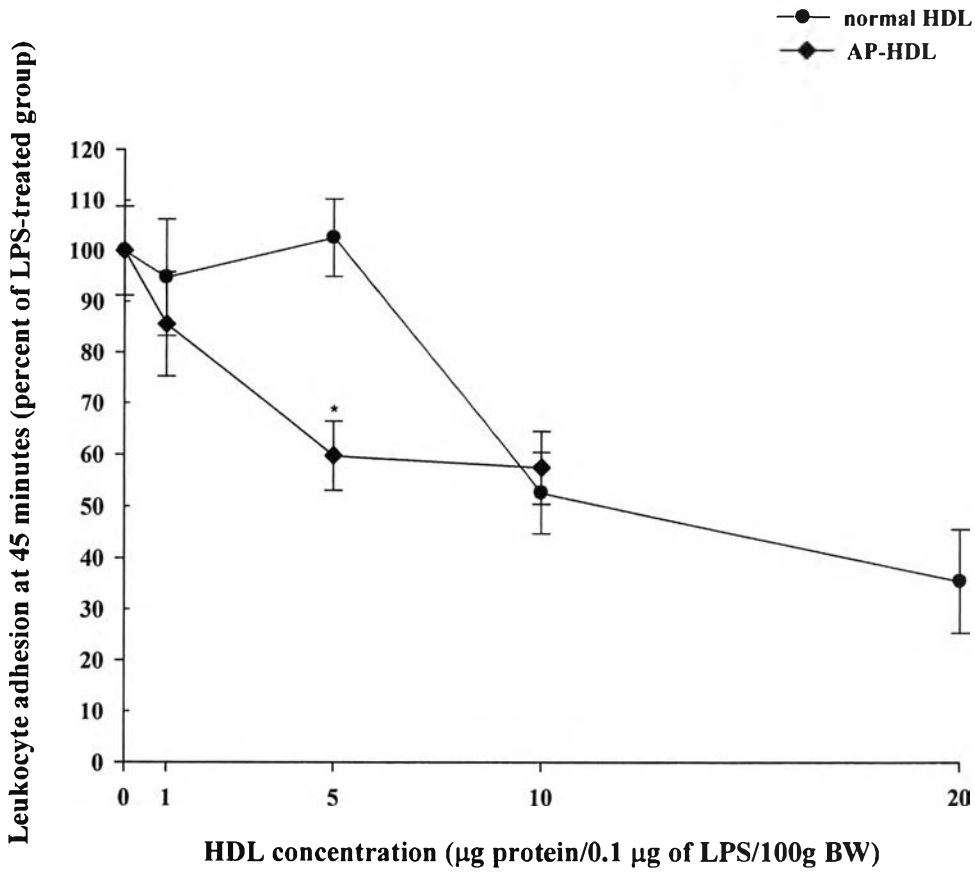

Figure 27 **Effects of various concentrations of HDL on LPS-induced leukocyte adhesion on endothelial cells. LPS was preincubated with NSS or normal HDL (—●—) or AP-HDL (—◆—) with increasing concentrations of HDL as indicated in the abscissa at 37^{O}C for 3 hours. After i.v. administration, leukocyte 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 number of leukocyte adhesion of LPS+normal HDL or LPS+AP-HDL group. Z represented number of leukocyte adhesion of LPS-treated group.**

***P<0.05 : significant difference from normal HDL group.**

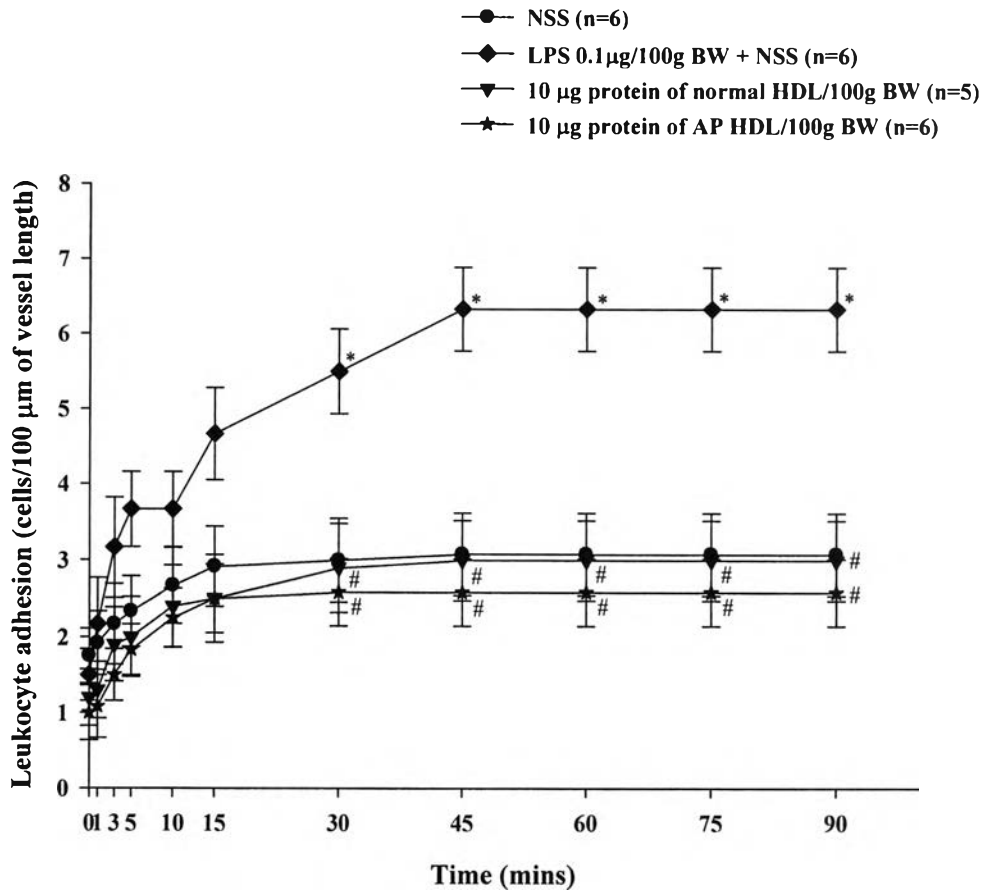

Figure 28 **Effects of LPS and HDL on LPS-induced leukocyte adhesion on endothelial cells. NSS (—●—), LPS (—◆—) or HDL (—▼—, —★—) was preincubated at 37^{O}C for 3 hours. After i.v. administration, leukocyte adhesion on endothelial cells of the mesenteric venules was counted as described in materials and methods.**

*P<0.05 : significant difference from NSS group.
$^{\#}$P<0.05 : significant difference from LPS group.

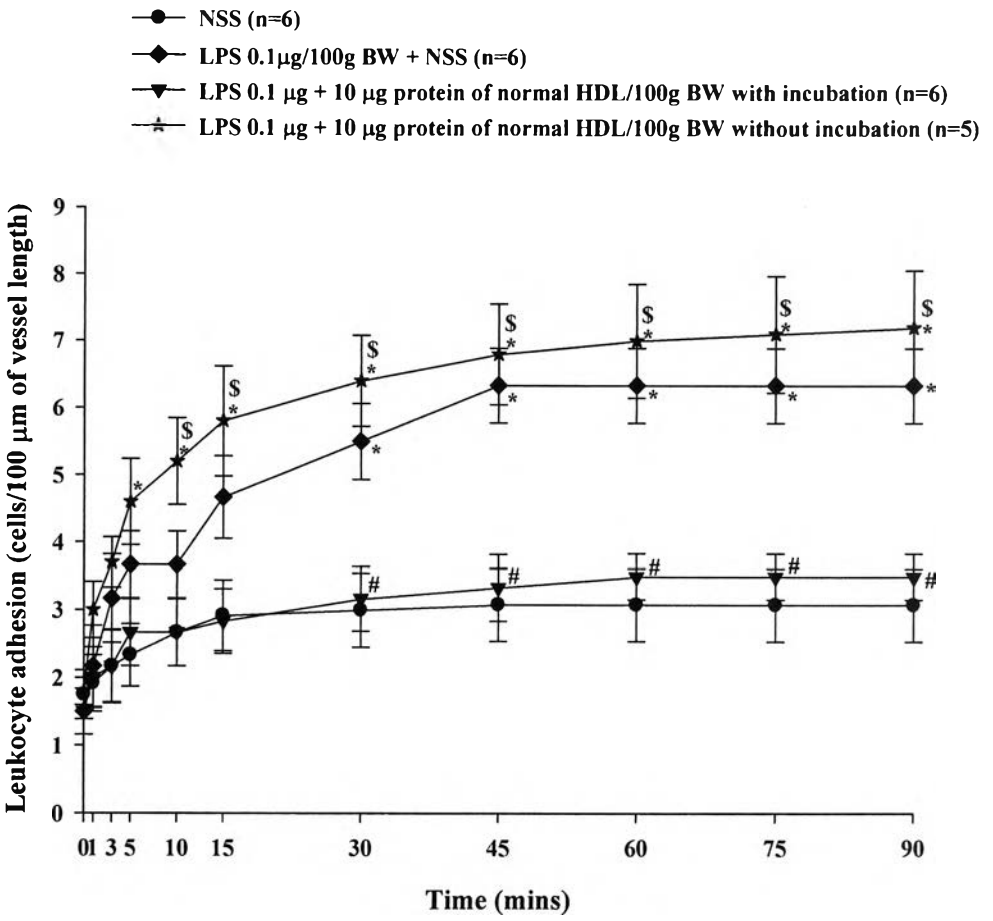

Figure 29 **Effects of incubation between LPS and normal HDL on LPS-induced leukocyte adhesion on endothelial cells. NSS was preincubated for NSS-treated group (—●—) and LPS was preincubated with NSS for LPS-treated group (—◆—) at 37^{O}C for 3 hours. LPS was (—▼—) or was not preincubated with normal 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 : significant difference from NSS group.
$^{\#}$P<0.05 : significant difference from LPS group.
$^{\$}$P<0.05 : significant difference from LPS+normal HDL with incubation group.

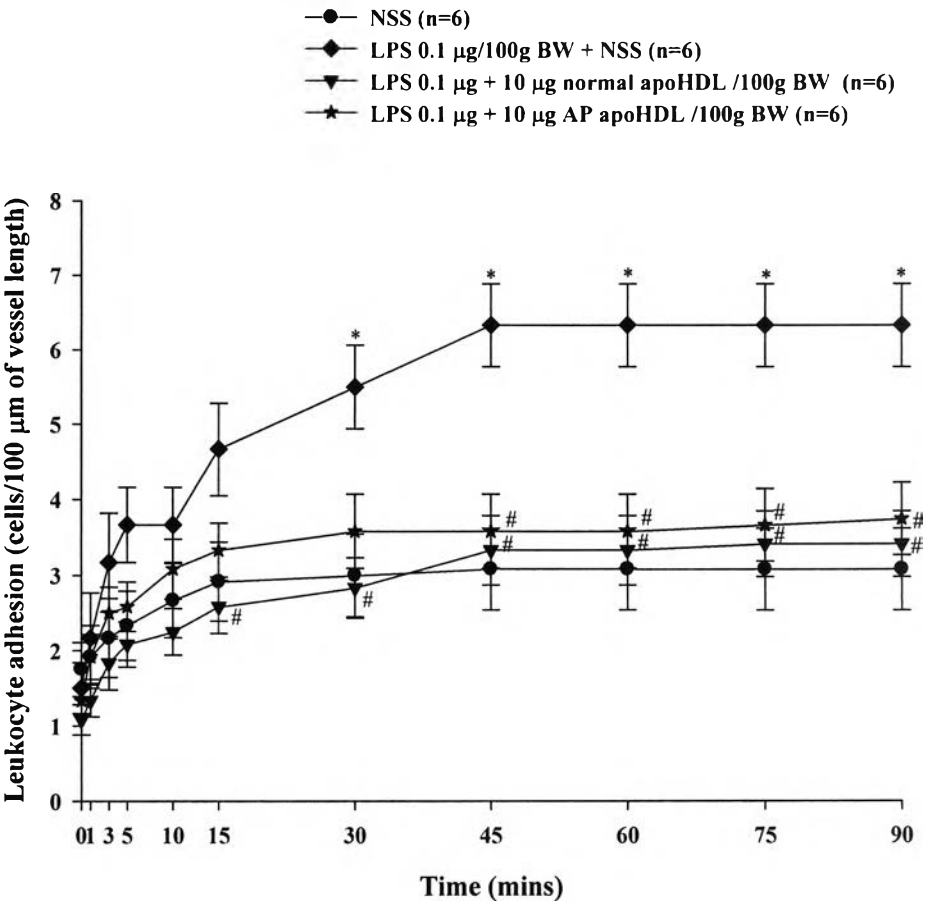

Figure 30 **Effects of apoHDL on LPS-induced leukocyte adhesion on endothelial cells. For NSS-treated group (—●—), NSS was preincubated at 37°C for 3 hours. LPS was preincubated with NSS for LPS-treated group (—◆—) or apoHDL for LPS+apoHDL-treated group (—▼—,—★—) at 37°C for 3 hours. After i.v. administration, leukocyte adhesion on endothelial cells of the mesenteric venules was counted as described in materials and methods.**

***P<0.05 : significant difference from NSS group.**
#P<0.05 : significant difference from LPS group.

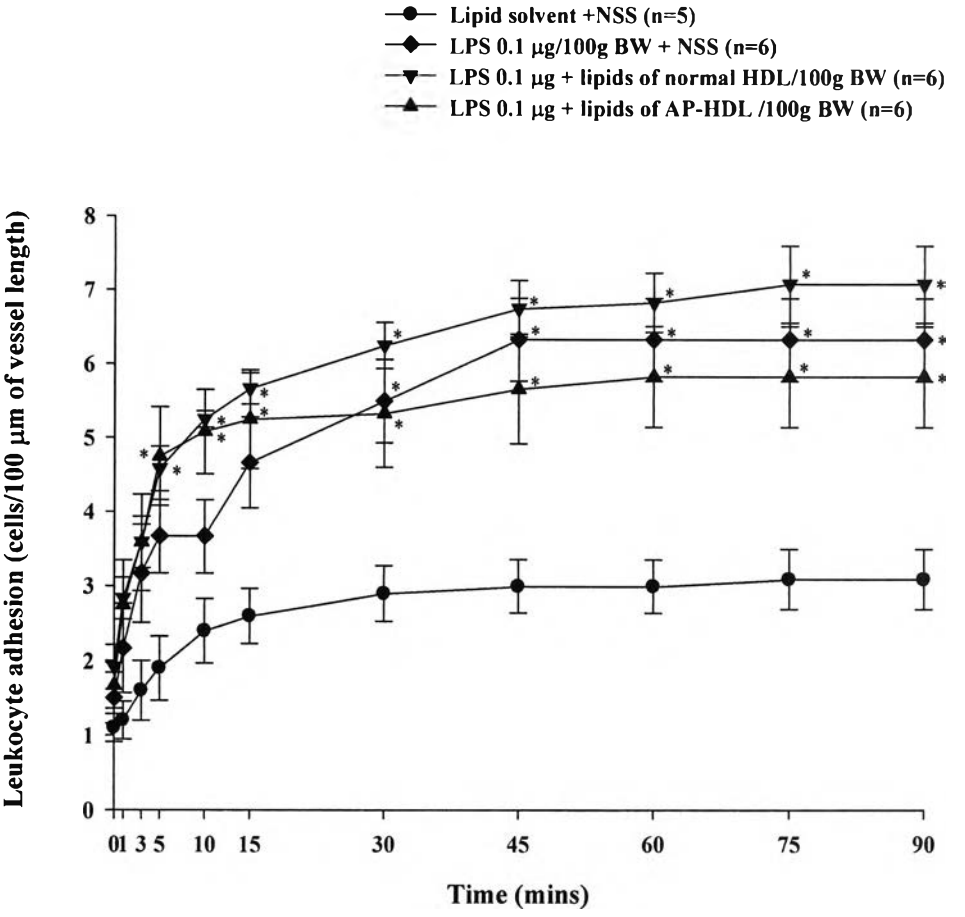

Figure 31 **Effects of lipids of HDL on LPS-induced leukocyte adhesion on endothelial cells. For lipid solvent-treated group (—●—), lipid solvent was preincubated with NSS at 37^{O}C for 3 hours. LPS was preincubated with NSS for LPS-treated group (—◆—) or lipids of HDL for LPS+lipids of HDL-treated 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 : significant difference from lipid solvent group.**

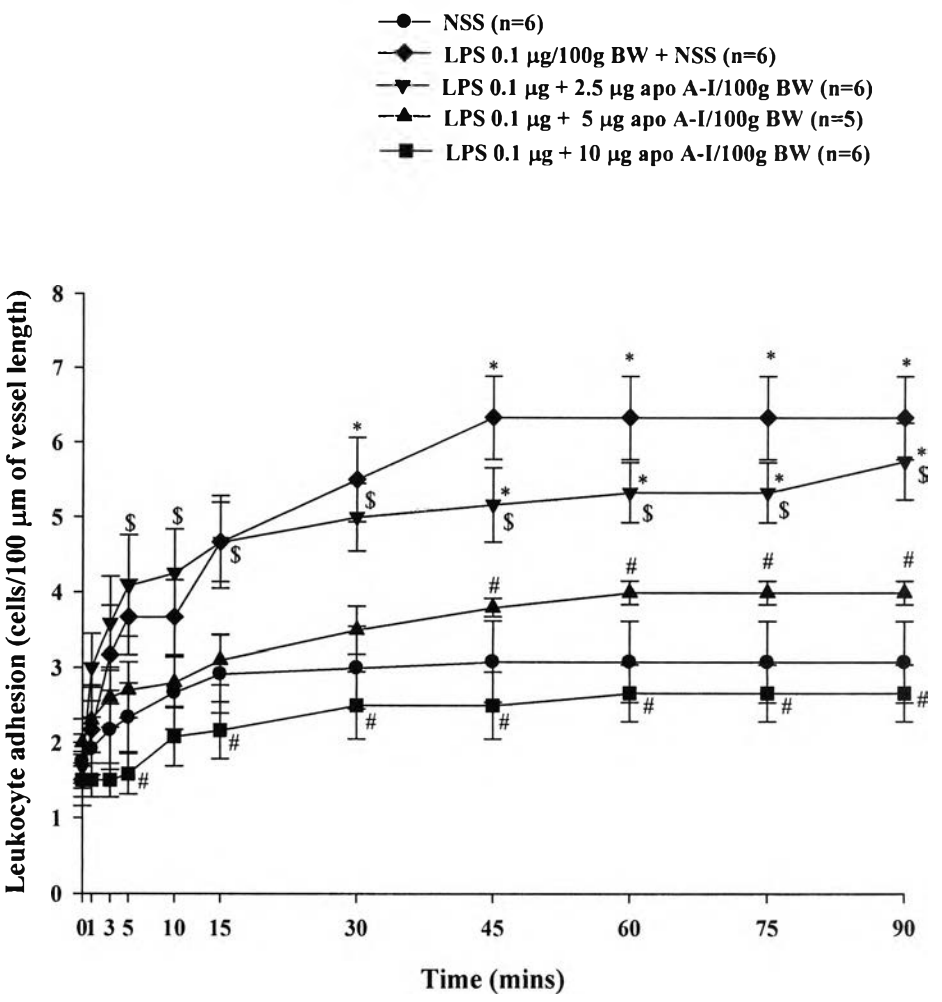

Figure 32 **Effects of apo A-I on LPS-induced leukocyte adhesion on endothelial cells. For NSS-treated group (—●—), NSS was preincubated at 37^{O}C for 3 hours. LPS was preincubated with NSS for LPS-treated group (—◆—) or apo A-I for LPS+apo A-I-treated group (—▼—, —▲—, —■—) at 37^{O}C for 3 hours. After i.v. administration, leukocyte adhesion on endothelial cells of the mesenteric venules was counted as described in materials and methods.**

*** P<0.05 : significant difference from NSS group.**
 # P<0.05 : significant difference from LPS group.
$ P<0.05 : significant difference from LPS+10 μg apo A-I group.

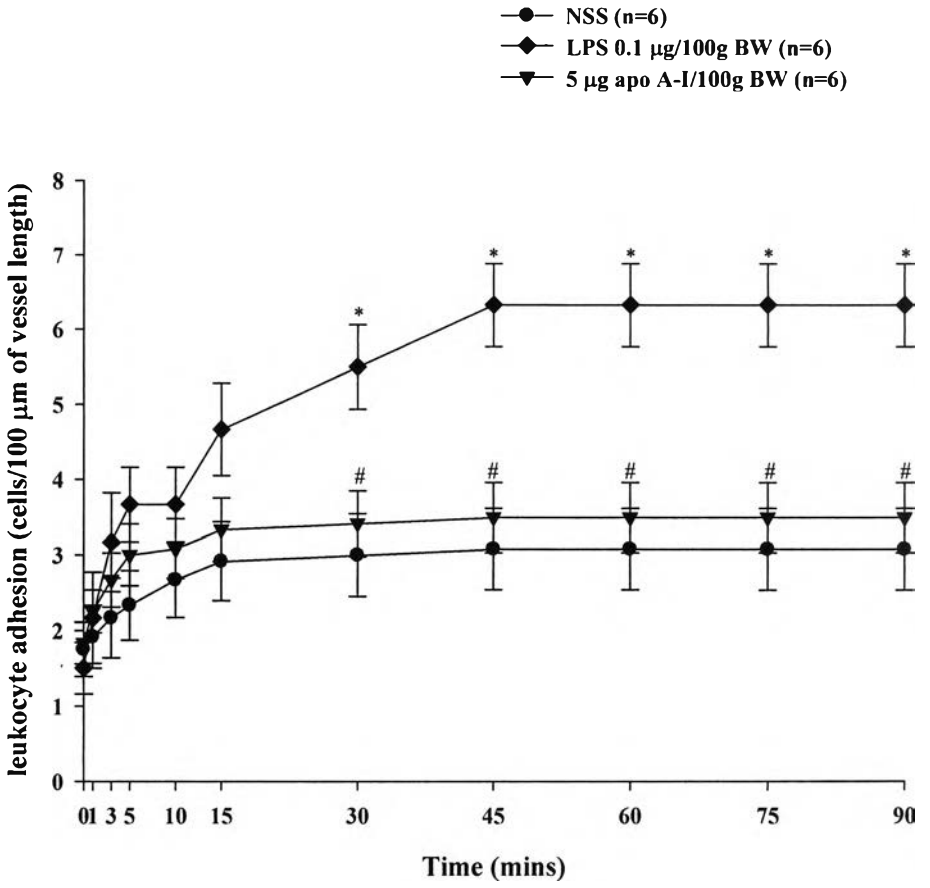

Figure 33 **Effects of LPS and apo A-I on leukocyte adhesion on endothelial cells. NSS (—●—), LPS (—◆—) or apo A-I (—▼—) was preincubated at 37°C for 3 hours. After i.v. administration, leukocyte adhesion on endothelial cells of the mesenteric venules was counted as described in materials and methods.**

*** P<0.05 : significant difference from NSS group.**
 # P<0.05 : significant difference from LPS group.

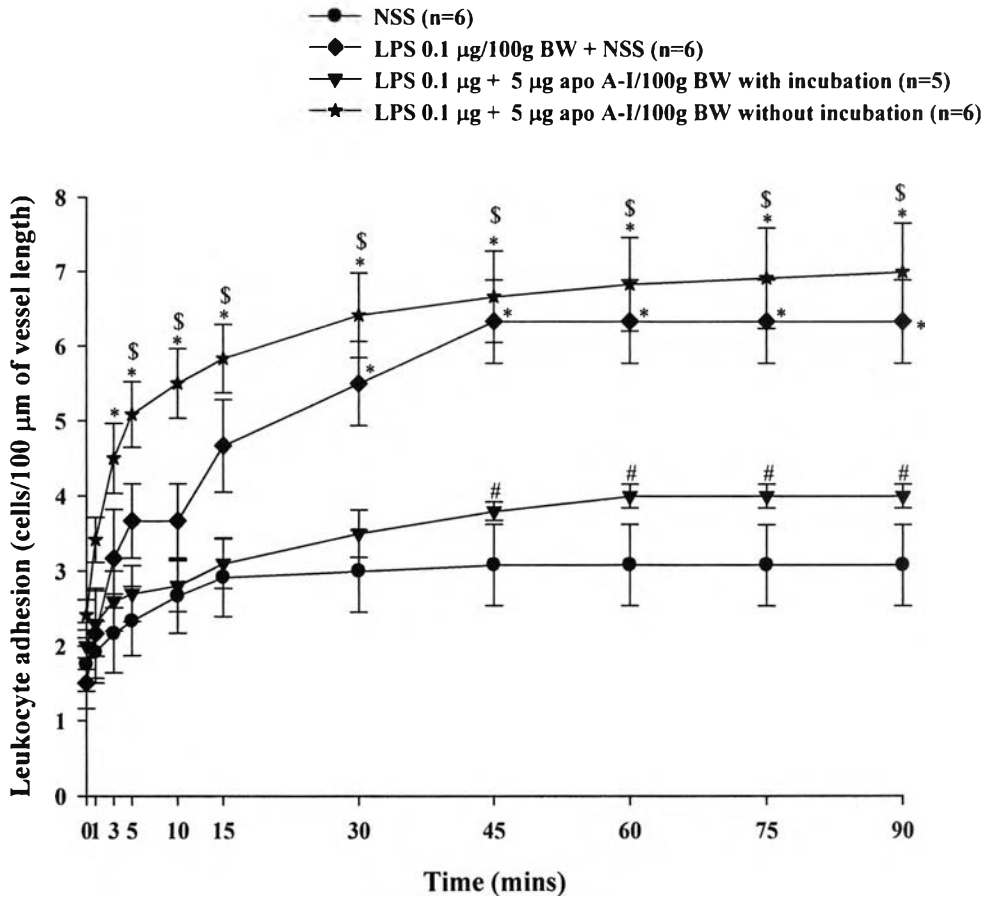

Figure 34 **Effects of incubation between LPS and apo A-I on LPS-induced leukocyte adhesion on endothelial cells. NSS was preincubated for NSS-treated group (—●—) and LPS was preincubated with NSS for LPS-treated group (—◆—) at 37^{O}C for 3 hours. LPS was (—▼—) or was not preincubated with apo A-I (—★—) 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 : significant difference from NSS group.**
 # P<0.05 : significant difference from LPS group.
$ P<0.05 : significant difference from LPS+apo A-I with incubation group.