Effect of L-NAME on the Synthesis of Plasma Fibrinogen in Mice

Yasutaka Takinishi1, Masako Okazaki1*, and Katsuji Oguchi1

1Department of Pharmacology, Showa University School of Medicine, Tokyo 142-8555, Japan

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Abstract. The effect of nitric oxide (NO) on plasma fibrinogen was investigated by treating mice with oral Nω-nitro-L-arginine-methyl ester (L-NAME), a non-selective NO synthase (NOS) inhibitor. Treatment with L-NAME significantly increased the concentration of plasma fibrinogen and hepatic expression of the α- and β-chains of fibrinogen, whereas plasma NO$_2^-$ level and hepatic expression of endothelial NOS (eNOS) mRNA were significantly decreased. These results suggest that L-NAME treatment can increase plasma fibrinogen levels via an increase in expression of fibrinogen mRNA in the liver, and NO may be involved in plasma fibrinogen increase.

Keywords: fibrinogen, Nω-nitro-L-arginine-methyl ester, mRNA
A increase in Plasma Fibrinogen by L-NAME. The RT reaction was performed in a 50-µl reaction mixture containing 2.5 µg of total liver RNA, 250 U moloney murine leukemia virus (M-MLV) reverse transcriptase (ReverTra Ace®, TOYOBO Co., Ltd., Osaka), 62.5 pM random primer, and all four deoxynucleotide phosphates (dNTPs: 10 mM each) in PCR buffer, pH 8.3, consisting of 75 mM KCl, 50 mM Tris-HCl, 3 mM MgCl₂, and 50 U RNase inhibitor. Samples were incubated at 42°C for 20 min, heated at 99°C for 5 min, and then the RT reaction was stopped. cDNA was amplified in 25 µl of PCR reaction mixture containing 5 µl cDNA, 0.4 µM of each primer, 2 µl of each dNTP (1 mM), 1 U AmpliTaq DNA polymerase (Sigma-Aldrich), and PCR buffer of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), and 1.5 mM MgCl₂.

Figure 1 shows the standard concentration curves for β-actin, prothrombin, eNOS, and α-, β-, and γ-fibrinogen. PCR products for β-actin and target RNA were quantified by measurement of fluorescence intensity. PCR of α-chain fibrinogen mRNA was performed with a Takara PCR thermal cycler (Takara Shuzo, Tokyo) for 21 cycles at 95°C for 60 s, 60°C for 60 s, and 72°C for 120 s. For β-chain fibrinogen mRNA, we used 22 cycles of 95°C for 60 s, 60°C for 60 s, and 72°C for 120 s; for γ-chain fibrinogen mRNA, 21 cycles of 94°C for 30 s, 53°C for 30 s, and 72°C for 60 s; for prothrombin mRNA, 22 cycles of 94°C for 60 s, 55°C for 60 s, and 72°C for 120 s; for eNOS mRNA, 30 cycles of 95°C for 60 s, 63°C for 60 s, and 72°C for 120 s. As an internal standard, β-actin mRNA was amplified from a cDNA template and detected using 25-cycle PCR with cycle settings of 94°C for 30 s, 60°C for 30 s, and 72°C for 60 s. A 10-µl volume from each reaction was resolved by 2% agarose gel electrophoresis. The level of target RNA from the liver of C57BL/6J mice was expressed as a ratio to that of β-actin RNA. Data are expressed as the means ± S.E.M. Analysis of variance

![Figure 1](image_url)

**Fig. 1.** Fluorescence intensity of RT-PCR reactions with time. The longitudinal axis shows fluorescence intensity and horizontal axis shows RT-PCR cycle number. Each electropherogram set is the representative of 4 mice. A: β-actin, B: prothrombin, C: eNOS, D: α-chain of fibrinogen, E: β-chain of fibrinogen, F: γ-chain of fibrinogen.
(ANOVA) following by the Tukey’s post-hoc test was performed to assess differences between the relevant control and each experimental group. Differences with $P$ values of less than 0.05 were considered to be significant.

A previous study showed that treatment for 7 days with L-NAME elevated plasma fibrinogen concentration in rats, but that treatment for 4 weeks with L-NAME did not cause chronic hyperfibrinogemia (8, 9). Here, plasma fibrinogen concentrations were significantly increased in mice treated with L-NAME for only 3 days ($333.9 \pm 15.6$ mg/dl at 300 mg/kg per day and $334.3 \pm 21.3$ mg/dl at 500 mg/kg per day) compared to controls ($237.0 \pm 7.9$ mg/dl), while they were significantly decreased in mice treated with L-arginine compared to 500 mg/kg L-NAME (Fig. 2). Treatment with 500 mg/kg L-NAME lead to a significant decrease in plasma NO$_2^-$ levels (data not shown), as determined by NO$_2^-$/NO$_3^-$ assay kit-F (Wako Pure Chemical Ind. Ltd., Osaka). The hepatic expression of eNOS mRNA was also significantly decreased in mice treated with 500 mg/kg L-NAME (54.8 $\pm$ 8.1%) compared to controls and increased significantly in arginine-treated mice (108.4 $\pm$ 24.4%) compared to 500 mg/kg L-NAME. Therefore, it is possible to say that 500 mg/kg L-NAME inhibits the hepatic expression of eNOS mRNA, decreases plasma NO level and then increases plasma fibrinogen concentration. We also investigated plasma levels of interleukin 6, a marker of acute vascular inflammation (10), and aspartate aminotransferase, a marker of cell membrane injury, using commercially available kits, but found no differences between control and treated animals (data not shown).

We previously reported that hepatic mRNA expression of each of the fibrinogen chains was significantly enhanced with an increase in plasma fibrinogen (11). The effect of L-NAME on the hepatic expression of $\alpha$, $\beta$, and $\gamma$-fibrinogen is not known. Treatment of mice with 500 mg/kg of L-NAME led to a significant increase in the levels of expression of hepatic $\alpha$-fibrinogen mRNA (142.1 $\pm$ 18.5%) and $\beta$-fibrinogen mRNA (131.9 $\pm$ 7.1%) (Fig. 3). In contrast, treatment with 500 mg/kg of L-arginine significantly decreased hepatic $\alpha$-fibrinogen mRNA (64.2 $\pm$ 3.3%), $\beta$-fibrinogen mRNA (90 $\pm$ 4.2%), and $\gamma$-fibrinogen mRNA (76.6 $\pm$ 4.4%) compared to 500 mg/kg L-NAME (Fig. 3). The hepatic expression of prothrombin mRNA was also significantly increased in mice treated with 500 mg/kg L-NAME (128.5 $\pm$ 9.7%) compared to controls and significantly decreased in arginine-treated mice (75.2 $\pm$ 3.9%) compared to 500 mg/kg L-NAME (Fig. 3).

This study therefore showed that short-term treatment with high-dose L-NAME significantly increased plasma fibrinogen concentration, with an increase in the hepatic expression of mRNA of the $\alpha$- and $\beta$-fibrinogen chains, whereas treatment with L-arginine led to a mild decrease in the hepatic mRNA of $\alpha$, $\beta$, and $\gamma$-fibrinogen expression. The initial steps in fibrinogen assembly involve the formation of $\alpha-\gamma$ and $\beta-\gamma$ dimers bound by disulfide bonds. Then a $\beta$-chain or $\alpha$-chain is added to each dimer and $\alpha-\beta-\gamma$ half molecules are formed. Finally, two half-molecules dimerize and become linked by five disulfide bonds to form the intact fibrinogen molecule (12). Roy et al. suggested that $\beta$-fibrinogen production is the rate limiting step of fibrinogen production (13), and therefore, the overexpression of $\beta$-fibrinogen in mice treated with 500 mg/kg L-NAME that we observed may have led to an increase in plasma fibrinogen. In addition, the significant increase in expression of prothrombin mRNA in mice treated with 500 mg/kg L-NAME may also have increased the thrombotic risk (14), while L-arginine may have protected against thrombosis due to the decrease in hepatic mRNA expression of $\alpha$, $\beta$, and $\gamma$-fibrinogen and prothrombin. A diminished endothelium-derived NO has been considered to play an important role in the atherogenic processes due to the endothelial dysfunction such as vasoconstriction (15). In this paper, we showed the possibility of NO involvement in the increase in plasma fibrinogen that enhanced the platelet aggregation, blood viscosity, and fibrin deposition (3).

In conclusion, these results suggest that the inhibition of NO production by treatment with high dose L-NAME, a non-selective NO synthase inhibitor, may increase the
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Risk of cardiovascular disease, via increased hepatic mRNA expression of α- and β-fibrinogen chains, leading to increased plasma fibrinogen levels, and increased prothrombin levels.

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References


