# Nicotinic acid treatment shifts the fibrinolytic balance favourably and decreases plasma fibrinogen in hypertriglyceridaemic men

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**Background** Nicotinic acid in gram doses decreases cholesterol and triglyceride concentrations in plasma, but the effect on haemostatic function is not known.

**Methods** Twenty-three men with hypertriglyceridaemia were treated with 4 g nicotinic acid daily for 6 weeks. Tests for haemostatic function and serum lipoproteins were performed before and at the end of the period of treatment.

**Results** Treatment with nicotinic acid had the expected effect on lipoprotein concentrations: it reduced the serum concentrations of triglyceride and the three major density fractions of triglyceride (very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL)). The VLDL cholesterol concentration was reduced, but that of HDL cholesterol was increased (all $P<0.0001$). The lipoprotein(a) (Lp(a)) concentration decreased significantly ($P<0.01$). The total fibrinolytic activity was increased by nicotinic acid treatment as indicated by decreases in plasminogen activator inhibitor-1 activity from 34.3 to 23.8 U/ml ($P<0.01$) and in $\alpha_2$-antiplasmin activity from 1.10 to 0.97 U/ml ($P<0.01$). The plasma fibrinogen concentration decreased from 3.55 to 3.01 U/ml ($P<0.01$). Multivariate analysis showed that the changes in $\alpha_2$-antiplasmin and Lp(a) concentrations could explain 53% of the change in plasma fibrinogen, suggesting that increased plasmin mobilization could be responsible for the decrease in plasma fibrinogen.

**Conclusion** This study of hypertriglyceridaemic men has shown that long-term treatment with nicotinic acid not only corrects serum lipoprotein abnormalities, but also reduces the fibrinogen concentration in plasma and stimulates fibrinolysis.

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## Introduction

Treatment with nicotinic acid in gram doses has been shown to correct or improve virtually all plasma lipoprotein abnormalities from phenotype IIA to V, increased lipoprotein(a) (Lp(a)) levels, and low concentrations of high density lipoprotein (HDL) cholesterol [1–3]; it also has beneficial effects on cardiovascular disease outcome. Two studies [4,5] in which nicotinic acid treatment has been used have reported an association between treatment and reductions in both coronary heart disease (CHD) and ischaemic heart disease (IHD) mortality.

Prospective studies [6,7] have indicated a pivotal role for haemostatic factors in the development of cardiovascular disease, but little is known about the effects of nicotinic acid on haemostatic function. Early studies [8] showed an increased plasma fibrinolytic activity after acute administration of nicotinic acid, but no published studies have used up-to-date techniques to evaluate the effects of long-term treatment with nicotinic acid on fibrinolytic and other haemostatic mechanisms.

Recent studies [9,10] have shown links between lipoprotein metabolism and the coagulation system, particularly between increased triglyceride concentrations and decreased fibrinolytic activity. In the present study of hypertriglyceridaemic men, we focused on the interaction between triglyceride metabolism and haemostasis. One major question was whether the expected reduction in triglyceride-containing lipoproteins after long-term treatment with nicotinic acid would be accompanied by increased fibrinolytic activity. Furthermore, at present nicotinic acid is probably the most efficient agent known for reducing the independent risk factor Lp(a) [2,11,12]. It has been suggested [13] that Lp(a) competes with plasminogen and tissue plasminogen activator (t-PA) in binding to fibrin, because of the high amino acid homology of apolipoprotein(a) with plasminogen and t-PA, thus affecting the conversion of plasminogen to plasmin and subsequent degradation of fibrin. There has been no explanation as to how a decrease in Lp(a) as a result of treatment with nicotinic acid may relate to the changes in fibrinolysis.

Because fibrinogen has been implicated in CVD as an independent risk factor [6,7,14], it was also of interest to examine whether nicotinic acid treatment could reduce the concentration of fibrinogen.

## Patients and methods

### Patients

Twenty-three normoglycaemic men, who had been referred consecutively to the metabolic ward at the Karolinska Hospital because of diet-resistant hypertriglyceridaemia, participated in the study (Table 1); 16 had hypertriglyceridaemia and seven had combined hyperlipidaemia. The lipid abnormalities had not normalized 4 weeks after advice from a dietician to follow a modified form of the American Heart Association Step 1 diet. The subjects were informed of the study and their consent to participate was obtained. The study was approved by the local ethics committee at the Karolinska Hospital. None of the patients had taken any lipid-lowering medication during the 3 months before the study, and no major medical event or hospital stay had occurred during the 6 months before their referral. The participants were examined by two of the investigators (J.J. and A.A.-C.). Current medication was continued and remained unchanged throughout the study.

Treatment with nicotinic acid (Nicangin, Draco-Astra, Sweden) was started on the second day after a patient's admission to the ward: 0.25 g was given at lunch, 0.5 g at dinner and 0.5 g later in the evening. The dosage was gradually increased over 3 days to 1 g nicotinic acid four times daily. On day 5, the patient was discharged from the ward and asked to continue the treatment. After 6 weeks of treatment the participants returned for clinical control and blood sampling.

Blood samples were drawn between 0800 and 0900 h after the patient had fasted overnight, on day 2 after admission to the ward before starting treatment, and after 6 weeks of treatment with 1 g nicotinic acid four times daily.

### Laboratory procedures

*Haemostatic function tests*

Tissue plasminogen activator antigen was determined by a double-antibody enzyme-linked immunosorbent assay (ELISA) (product No 101120, Biopol AB, Umeå, Sweden). The inhibitor of t-PA plasminogen activator inhibitor (PAI)-1 was determined by a functional spectrophotometric method [15] using kits (Biopool AB). It should be noted that, because t-PA was analysed immunochemically, both free t-PA and (to some extent) t-PA included in any t-PA–PAI-1 complexes were measured. Plasma fibrinogen was analysed immunochemically by a quantitative electroimmunoassay (rocket electrophoresis) and $\alpha_2$-antiplasmin was determined by a spectrophotometric method [16]. Analysis of the von Willebrand factor was performed immunochemically by ELISA (Diagnostica Stago, Asniér, France).

Reference values defined as mean ± 2SD were provided by the manufacturer for the t-PA, PAI-1 and $\alpha_2$-antiplasmin methods. The reference values for fibrinogen and von Willebrand factor were based on those measured in 75 and 34 apparently healthy men and women, respectively.

**Table 1 Characteristics of the 23 hypertriglyceridaemic men at the time of the metabolic study**

| Characteristics | No. of subjects or mean (min–max) |
|---|---|
| Age (years) | 47 (27–67) |
| Body mass index (kg/m$^2$) | 27.3 (21.4–32.0) |
| Present smoker | 9 |
| Coronary heart disease | |
| Myocardial infarction | 3 |
| Coronary bypass surgery | 1 |
| Angina pectoris | 2 |
| Asymptomatic and no medication | 13 |
| Lipoprotein phenotype | |
| Combined hyperlipidaemia | 7 |
| Hypertriglyceridaemia | 16 |
| Hypertension | 7 |
| β-Blocker medication | 7 |

*Comparison of analyses of blood coagulation components in citrated and EDTA blood*

Haemostatic variables were analysed using disodium-EDTA plasma that had been frozen for up to 2 years at –70°C. Because these variables are usually analysed using trisodium citrate plasma, a comparison was performed. Venous blood samples were taken simultaneously from three healthy individuals and introduced into trisodium citrate (0.129 mol/l, 0.5 ml + 4.5 ml blood) and $K_3EDTA$ (0.47 mol/l, 0.05 ml + 4.95 ml blood), the latter consistent with what was used in the trial. Frozen plasmas were analysed for t-PA, PAI-1, von Willebrand factor, $\alpha_2$-antiplasmin and fibrinogen. The values obtained for the five haemostatic function tests analysed were essentially the same whether the analysis used EDTA or citrate plasma, after correction for the differences in dilution.

A possible bias of results related to the duration of plasma storage was tested for the haemostatic variables and Lp(a) by comparing the values from the first 11 patients (plasma storage time 1–2 years) with those of the last 12 (plasma storage time less than 1 year). The former group comprised three patients with combined hyperlipidaemia and eight with hypertriglyceridaemia, and the latter group comprised four patients with combined hyperlipidaemia and eight with hypertriglyceridaemia. Values at baseline, after treatment and for the change in concentrations produced by treatment for the haemostatic variables and Lp(a) differed very little and were statistically non-significant between the two groups, indicating no effect of plasma storage.

*Lipoproteins*

Serum lipoproteins, with the exception of Lp(a), were analysed on fresh serum from blood that had been allowed to clot at room temperature for 2 h. To the serum were added $Na_2EDTA$ (1.3 mmol/l) and merthiolate (0.25 mmol/l). Very low density lipoproteins (VLDL), low density lipoproteins (LDL) and HDL were separated by a combination of ultracentrifugation and precipitation techniques as described previously [17] and the concentrations of cholesterol [18] and triglyceride [19] in the lipoprotein

fractions were determined. This technique also enabled accurate estimation of VLDL and LDL levels in hypertriglyceridaemia.

Lp(a) was assayed on one occasion, in serum that had been stored at –70°C, using a commercial immunoradiometric assay (Pharmacia Diagnostics AB, Sweden).

Blood glucose and serum albumin were measured in fresh blood using an enzymatic (glucose oxidase) method (Kodak Ektachem, Germany).

### Statistical analysis

Values are given as mean values with standard deviations or minimum and maximum values. Treatment effects were tested for significance using two-tailed paired Student's t-test. Individual values of skewed variables were transformed by the natural logarithm and tested for normal distribution before being entered in univariate correlation analysis and multiple stepwise regression analysis. Because of the high number of correlation analyses, only coefficients with a value of $R > 0.53$, corresponding to $P < 0.01$, were considered significant in the univariate calculations. The univariate and multiple analyses were performed on three sets of data: baseline values, value after treatment, and change in concentrations.

In the multiple stepwise regression analysis, we tested the independent correlation of various parameters with the concentrations of variables of haemostasis. The variable with the greatest partial correlation coefficient was entered at each step until no variable remained that had a Scheffé F-value 'to enter' of 4 or more. Because only 23 subjects participated in the study, multivariate analysis was structured as two models, restricted to five and six variables respectively, in order to retain the power of the statistical analysis. The first model aimed to elucidate the independent predictive power of the major lipoprotein classes on the concentration of variables of haemostasis. Thus VLDL cholesterol, LDL cholesterol, HDL cholesterol and Lp(a) were included as independent variables and each haemostatic function variable was consecutively included as the dependent one. The second multivariate model aimed to detect independent predictors within the haemostasis system. The five haemostasis variables in turn were selected as the dependent variable, with the remaining four and Lp(a) as the independent variables.

## Results

On average, the patients lost 1.2 kg in weight during the 6 weeks of treatment, resulting in a significant decrease in body mass index (BMI) from 27.3 (SD 3.2) kg/m$^2$ to 26.9 (3.0) kg/m$^2$ ($P < 0.05$). The decrease in BMI was non-significantly correlated to the changes in lipoprotein concentration and measures of haemostatic function.

Mean fasting glucose concentration increased from 5.4 (0.6) mmol/l to 5.6 (0.6) mmol/l ($P < 0.02$) during the period of treatment. The change was non-significantly correlated both to changes in VLDL, LDL and HDL cholesterol, and to changes in haemostatic function tests. The mean concentration of albumin did not change, being 42 g/l both before and after treatment with nicotinic acid.

### Haemostatic function

The mean percentage coefficient of variation (SD/mean) was calculated from duplicate samples, yielding the following values: PAI-1 11%; t-PA 12%; $\alpha_2$-antiplasmin 7%; von Willebrand factor 13%; fibrinogen 6%.

The mean values for the variables of haemostasis before and after nicotinic acid treatment fell within the reference intervals of the Coagulation Laboratory at the Karolinska Hospital for fibrinogen, $\alpha_2$-antiplasmin and the von Willebrand factor (Table 2). In contrast, the average concentrations of PAI-1 were clearly above the reference interval both before and after treatment, and the mean t-PA values before treatment were on the upper border line of the reference interval.

There was a significant reduction in the plasma concentrations of the three fibrinolytic variables and fibrinogen, whereas that of von Willebrand factor increased (Table 2). The ratio between PAI-1 and t-PA values was essentially unchanged by treatment, as judged by the mean values before and after treatment.

### Lipoproteins

Serum cholesterol and triglyceride concentrations were, as expected, reduced by treatment with nicotinic acid (Table 3). Triglyceride concentrations were reduced in all three major density fractions, whereas VLDL cholesterol was reduced, LDL cholesterol was unchanged and the HDL fraction was increased; the Lp(a) concentration was decreased by treatment with nicotinic acid (Table 3).

### Relationships between serum lipoprotein levels and haemostatic function

Univariate analysis showed correlations between serum triglycerides or (Table 4) VLDL cholesterol with PAI-1, at baseline and after treatment.

In the multivariate model with PAI-1 as the dependent variable and lipids as independent variables, at baseline

**Table 2 Haemostatic function values before and after 6 weeks of nicotinic acid treatment in 23 hypertriglyceridaemic men**

| | Baseline | After treatment |
|---|---|---|
| Plasminogen activator inhibitor-1 (U/ml) (ref. < 15 U/ml)[a] | 34.3 (18.0) | 23.78 (17.1)** |
| Tissue plasminogen activator antigen (U/ml) (ref. 2.3–13.2 U/ml)[a] | 15.2 (3.7) | 12.9 (3.1)*** |
| $\alpha_2$-Antiplasmin (U/ml) (ref. 0.80–1.20 U/ml)[a] | 1.10 (0.17) | 0.97 (0.17)** |
| Von Willebrand factor (U/ml) (ref. 0.5–1.50 U/ml)[a] | 0.81 (0.33) | 0.97 (0.48)* |
| Fibrinogen (g/l) (ref. 2.1–4.2 g/l)[a] | 3.55 (0.56) | 3.01 (0.81)** |

Values are expressed as mean (SD). [a]Reference values according to text, see methods. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (paired Student's t-test).

**Table 3 Lipoprotein concentrations before and after 6 weeks of treatment with nicotinic acid in 23 hypertriglyceridaemic men**

| | Baseline | After treatment |
|---|---|---|
| Lipids (mmol/l) | | |
| Serum cholesterol | 7.91 (1.45) | 6.40 (1.44)*** |
| Serum triglyceride | 5.24 (2.91) | 2.86 (1.71)*** |
| VLDL cholesterol | 2.02 (0.95) | 0.87 (0.50)*** |
| VLDL triglyceride | 4.06 (2.63) | 2.09 (1.46)*** |
| LDL cholesterol | 4.82 (1.41) | 4.36 (1.42) |
| LDL triglyceride | 0.68 (0.18) | 0.47 (0.15)*** |
| HDL cholesterol | 0.88 (0.15) | 1.12 (0.26)*** |
| HDL triglyceride | 0.18 (0.06) | 0.15 (0.06)* |
| Lipoprotein(a) (mg/l) | 369 (414) | 262 (287)** |

Values are expressed as mean (SD). VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein. *$P<0.05$; **$P<0.01$; ***$P<0.001$ (paired Student's t-test).

**Table 4 Univariate correlations between lipoproteins and haemostatic function levels at baseline, after 6 weeks administration of nicotinic acid 4 g daily, and calculated on change in concentration.**

| | Cholesterol | | | |
|---|---|---|---|---|
| | VLDL | LDL | HDL | LP(a) |
| At baseline | | | | |
| PAI-1 | 0.49 | −0.55* | 0.17 | 0.01 |
| t-PA | 0.59* | −0.27 | 0.43 | 0.04 |
| $\alpha_2$-AP | −0.20 | −0.34 | 0.39 | −0.30 |
| VWF | −0.29 | −0.38 | −0.01 | −0.23 |
| Fibrinogen | −0.16 | −0.30 | −0.39 | 0.03 |
| After treatment | | | | |
| PAI-1 | 0.66* | 0.14 | 0.17 | −0.16 |
| t-PA | 0.31 | −0.32 | 0.10 | −0.00 |
| $\alpha_2$-AP | 0.01 | 0.31 | 0.13 | −0.01 |
| VWF | −0.30 | −0.30 | −0.25 | −0.27 |
| Fibrinogen | −0.29 | 0.49 | 0.14 | −0.18 |
| Change in concentration | | | | |
| PAI-1 | 0.21 | −0.08 | −0.26 | −0.17 |
| t-PA | 0.37 | −0.21 | −0.12 | −0.04 |
| $\alpha_2$-AP | 0.01 | 0.67* | −0.05 | 0.02 |
| VWF | 0.13 | −0.42 | −0.45 | −0.39 |
| Fibrinogen | −0.08 | 0.46 | 0.18 | 0.50 |

VLDL, very low-density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; Lp(a), lipoprotein(a); PAI-1, plasminogen activator inhibitor type 1; t-PA, tissue-plasminogen activator; $\alpha_2$-AP, $\alpha_2$-antiplasmin; VWF, von Willebrand factor. Pearson correlation coefficients: *$P<0.01$; $R>0.53$.

only LDL cholesterol showed a significant correlation, explaining 27% of the PAI-1 concentration (adjusted $R^2 = 0.27$; Table 5). After treatment, calculations showed that VLDL cholesterol correlated with PAI-1 (adjusted $R^2 = 0.35$; Table 5). Changes in PAI-1 concentrations were not correlated with changes in lipoprotein concentrations, in either univariate or multivariate analysis (Tables 4 and 5).

At baseline, t-PA correlated with lipid concentrations in both univariate and multivariate analysis (Tables 4 and 5), but analyses of concentrations after treatment and induced changes in concentrations showed that there were no significant correlations (Tables 4 and 5).

The changes in $\alpha_2$-antiplasmin and LDL cholesterol concentrations were shown to be correlated significantly by both univariate analysis (Table 4) and multivariate analysis (adjusted $R^2 = 0.28$; Table 5).

**Table 5 Multiple stepwise regression with variables of the haemostatic function test, one at a time, as dependent variable (left column) and VLDL, LDL, HDL cholesterol and Lp(a), as independent variables (right column). The calculations were performed on the baseline values, values after treatment, and change in concentration produced by treatment**

| | |
|---|---|
| Calculations on baseline values | |
| PAI-1 | LDL-C: 0.27 |
| t-PA | VLDL-C: 0.20, +HDL-C: 0.42, + Lp(a): 0.55 |
| $\alpha_2$-AP | Lp(a): 0.15 |
| VWF | – |
| Fibrinogen | – |
| Calculations on after-treatment values | |
| PAI-1 | VLDL-C: 0.35 |
| t-PA | – |
| $\alpha_2$-AP | – |
| VWF | – |
| Fibrinogen | LDL-C: 0.20 |
| Calculations on change in concentrations | |
| PAI-1 | – |
| t-PA | – |
| $\alpha_2$-AP | LDL-C: 0.28 |
| VWF | HDL-C: 0.18, LDL-C: 0.37 |
| Fibrinogen | Lp(a): 0.22 |

The values denote the adjusted $R^2$ in the multivariate analysis. For further information see statistics. Abbreviations are as in Table 4.

In multiple stepwise regression analysis, the change in von Willebrand factor concentration was explained by the change in HDL cholesterol (adjusted $R^2 = 0.18$) and the change in LDL cholesterol (total adjusted $R^2 = 0.37$; Table 5).

In multivariate analysis, plasma fibrinogen concentration after treatment was correlated with LDL cholesterol concentration (adjusted $R^2 = 0.20$), and the change in fibrinogen concentration was predicted by the change in Lp(a) concentration (adjusted $R^2 = 0.22$; Table 5).

## Relationships between the variables of haemostatic function

Table 6 shows the univariate correlations between variables of haemostatic function at baseline, after treatment and as changes in concentration. Significant correlations ($P < 0.01$) were demonstrated between PAI-1 and t-PA at baseline and between plasma fibrinogen and $\alpha_2$-antiplasmin after treatment. Correlations between changes in concentrations of plasma fibrinogen and $\alpha_2$-antiplasmin were significant (Table 6).

The five variables of haemostasis and Lp(a) were also subjected to multiple stepwise regression analysis. With PAI-1 concentrations as the dependent variable, we found a significant contribution by t-PA at baseline (adjusted $R^2 = 0.34$), and after treatment (adjusted $R^2 = 0.17$; Table 7).

With t-PA as the independent factor, the findings for PAI-1 were mirrored: there was a significant association of t-PA with PAI-1 concentrations at baseline and after treatment (adjusted $R^2 = 0.17$; Table 7). The change in t-PA concentration could be explained by that in von Willebrand factor concentration (adjusted $R^2 = 0.21$; Table 7).

$\alpha_2$-Antiplasmin concentrations after treatment were correlated with those of fibrinogen (adjusted $R^2 = 0.37$)

**Table 6 Univariate correlations between haemostatic function levels at baseline, after 6 weeks administration of nicotinic acid 4 g daily, and calculated on change in concentration**

| | t-PA | $\alpha_2$-AP | VWF | Fibrinogen |
|---|---|---|---|---|
| At baseline | | | | |
| PAI-1 | 0.61* | 0.00 | −0.36 | −0.40 |
| t-PA | | 0.03 | −0.20 | −0.12 |
| $\alpha_2$-AP | | | 0.27 | −0.04 |
| VWF | | | | 0.31 |
| After treatment | | | | |
| PAI-1 | 0.46 | 0.14 | −0.24 | −0.16 |
| t-PA | | −0.04 | 0.20 | 0.01 |
| $\alpha_2$-AP | | | −0.31 | 0.64* |
| VWF | | | | 0.06 |
| Change in concentration | | | | |
| PAI-1 | 0.16 | −0.09 | 0.01 | −0.28 |
| t-PA | | −0.13 | 0.50 | 0.13 |
| $\alpha_2$-AP | | | | 0.58* |
| VWF | | | | −0.22 |

Abbreviations are as in Table 4. *$P<0.05$ (paired Student's t-test).

and von Willebrand factor (total adjusted $R^2 = 0.48$) in the multivariate analyses (Table 7). Calculations based on changes in concentrations showed a significant contribution of fibrinogen concentration to the $\alpha_2$-antiplasmin concentration (adjusted $R^2 = 0.30$; Table 7). There were no significant associations at baseline.

Change in von Willebrand factor concentration was independently correlated to the changes in concentration of t-PA (adjusted $R^2 = 0.21$) and Lp(a) (combined adjusted $R^2 = 0.32$) in multivariate analysis (Table 7).

Fibrinogen concentration after treatment could be predicted by the $\alpha_2$-antiplasmin concentration (adjusted $R^2 = 0.37$; Table 7), and change in its concentration was significantly correlated to the corresponding alterations in both $\alpha_2$-antiplasmin (adjusted $R^2 = 0.30$) and Lp(a) levels (combined adjusted $R^2 = 0.53$; Table 7).

## Discussion

Treatment of hypertriglyceridaemic men with nicotinic acid, in addition to producing the expected corrections of lipid levels, had a significant influence on haemostatic variables that would be compatible with producing enhanced fibrinolysis and a substantial decrease in plasma fibrinogen concentration.

Two points should be mentioned before these findings are discussed. The first concerns the design of the study. Because of the inherent and almost unavoidable side-effects of nicotinic acid, we decided to perform an open study without a parallel control group. When starting their nicotinic acid treatment, almost all the patients experience flushing and sensation of heat, particularly in the face. For this reason it was not possible to perform a double-blind, randomized placebo-control study. Instead, we compared our findings with regard to the effects of treatment with nicotinic acid on lipids, with those of previous studies. We considered this design to be appropriate to a hypothesis-generating study of nicotinic acid treatment. The effects of

**Table 7 Multiple stepwise regression with a haemostatic function test as dependent variable (left column) and the remaining ones, together with Lp(a), as independent variables (right column)**

| | |
|---|---|
| Calculations on baseline values | |
| PAI-1 | t-PA: 0.34 |
| t-PA | PAI-1: 0.34 |
| $\alpha_2$-AP | – |
| VWF | – |
| Fibrinogen | – |
| Calculations on after treatment values | |
| PAI-1 | t-PA: 0.17 |
| t-PA | PAI-1: 0.17 |
| $\alpha_2$-AP | Fibrinogen: 0.37, VWF: 0.48 |
| VWF | – |
| Fibrinogen | $\alpha_2$-AP: 0.37 |
| Calculations on change in concentrations | |
| PAI-1 | – |
| t-PA | VWF: 0.21 |
| $\alpha_2$-AP | Fibrinogen: 0.30 |
| VWF | t-PA: 0.21, Lp(a): 0.32 |
| Fibrinogen | $\alpha_2$-AP: 0.30, Lp(a): 0.53 |

The values denote the adjusted $R^2$ in the multivariate analysis. For further information see statistics. Abbreviations are as in Table 4.

nicotinic acid treatment on the serum concentrations of lipids that we observed matched previously published findings [1–3], lending support to the overall validity of our present results. Thus triglyceride concentrations were decreased in serum and in the VLDL, LDL and HDL fractions. Cholesterol was decreased in serum and the VLDL fraction, increased in the HDL fraction and unchanged in the LDL fraction. Lp(a) concentration was decreased in serum.

The second point concerns the blood material used for analysis of haemostatic factors. All analyses were performed on EDTA-plasma, rather than the citrated plasma that is used conventionally. A trial comparison gave essentially the same concentrations for the five haemostasis factors analysed, regardless of which plasma was used. Although the possibility cannot be excluded that analyses using the two different types of plasmas may yield minor differences in concentration, we do not believe that a systematic bias violated the overall results of the study.

The new findings of the present study are the nicotinic acid-induced stimulation of fibrinolysis and the decrease in plasma fibrinogen. It has been reported [8,20] that, in humans, short-term treatment with nicotinic acid (a single injection and one day of oral treatment) causes a release of plasminogen activator, but it has also been suggested [21] that this effect disappears during long-term treatment because of counter-regulation. In the present study, in which treatment with nicotinic acid was given for 6 weeks, we observed pronounced reductions in PAI-1 and $\alpha_2$-antiplasmin concentrations, indicating a shift of the regulatory balance in favour of the agonists of the fibrinolytic system. These findings provide clinical confirmation of a recently published *in vitro* study [22] that showed increased fibrinolysis by nicotinic acid through attenuation of PAI-1 synthesis in a hepatoma cell line.

The t-PA method used measures both free t-PA and the

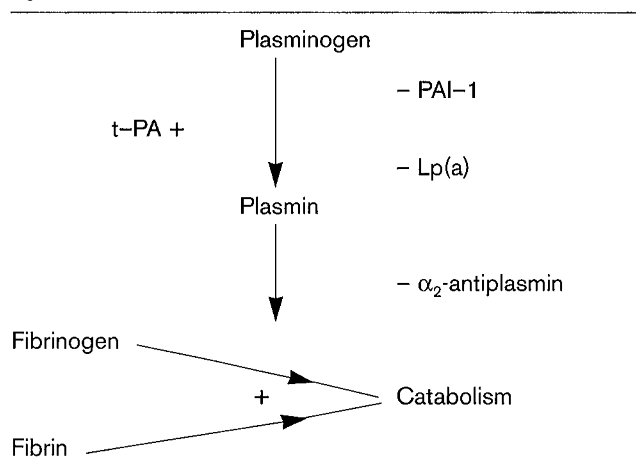
PAI-1-complex-bound t-PA and the decrease in t-PA levels is likely to be secondary to the PAI-1 reduction — a reasoning well supported by previous extensive documentation [23]. This assumption is also supported by the strong positive correlations between PAI-1 and t-PA observed in the present study.

The reduction in PAI-1 concentration results in a greater generation of plasmin, followed by rapid inhibition of plasmin as a result of the formation of a complex with $\alpha_2$-antiplasmin, and a consequent decreased $\alpha_2$-antiplasmin concentration in plasma. A similar effect of nicotinic acid on the fibrinolytic system has been suggested previously [24] in a study in which nicotinic acid was given as an infusion. The reduction in Lp(a) concentration as a result of treatment with nicotinic acid might also cause a more efficient conversion of plasminogen to plasmin, because of reduced competition by Lp(a) for the plasminogen–fibrin binding site [13].

The decrease in PAI-1 activity in plasma was not correlated to any alteration in lipid concentration, in either univariate or in multivariate analysis. In general, the strength of the correlations between triglyceride-rich lipoproteins and PAI-1 activity was weak. The only significant correlation that we found in multivariate analysis was between VLDL cholesterol and PAI-1 when concentrations after treatment were entered in the calculations. This finding is in contrast to previous notions of a causal link between triglyceride metabolism and PAI-1 activity. The clinical support for a metabolic link between triglycerides and PAI-1 has derived mainly from cross-sectional studies [9,10,25] in which there was no triglyceride-decreasing intervention. Selection of a hypertriglyceridaemic male population, as in the present study, narrows the ranges for serum triglyceride concentrations and plasma PAI-1 activity, making it more difficult to demonstrate correlations at baseline. However, treatment was associated with significant changes in both triglycerides and PAI-1. The decreases were substantial, and varied between individuals, thus providing a suitable numerical basis for the calculation of correlations between them. Our findings, although limited to a situation of nicotinic acid intervention, suggest that triglycerides are not critical in regulating PAI-1 concentrations in plasma.

The decrease in fibrinogen, which was of the same magnitude as that seen when a smoker gives up smoking [26] may be of clinical importance. The change in fibrinogen concentration in response to nicotinic acid treatment was not correlated to any alteration in lipoprotein concentration. The regulatory mechanisms for plasma fibrinogen and for the fibrinolytic system are not fully understood. As fibrinogen is an acute-phase protein, its synthesis is stimulated by cytokines such as interleukin (IL)-1, IL-6, interferon (INF)-$\alpha$ and tumour necrosis factor (TNF)-$\alpha$ [27]. Similarly, PAI-1 is an acute-phase reactant. Endothelial cell culture experiments [28] have shown that synthesis of PAI-1 is stimulated by IL-1-$\alpha$ and TNF-$\alpha$. A recent clinical study [29] has demonstrated a correlation between plasma PAI-1 and cytokine levels in patients with sepsis and those with disseminated intravascular coagulation. Whether nicotinic acid could directly or indirectly downregulate the synthesis of fibrinogen and PAI-1 is unknown, but experimentally in hamsters it was able to reduce the production of procollagens I and III induced by bleomycin [30]. The facts that concentrations of the von Willebrand factor, which also is an acute-phase reactant, increased during treatment, and that serum albumin concentrations were unchanged, indicate that the decreases in PAI-1 activity and plasma fibrinogen concentration are unlikely to be the result of 'general' reductions in inflammatory activity. The observed decreases in fibrinogen and PAI-1 concentrations could possibly be due to interference of nicotinic acid, either with the synthesis of specific stimulatory cytokines, or directly in the synthesis of both plasma proteins.

**Fig. 1**




In the hypothetical model, it is proposed that the decrease in plasminogen activator inhibitor-1 (PAI-1) and lipoprotein(a) (Lp(a)) concentrations produced by treatment with nicotinic acid (1 g four times a day) results in enhanced mobilization of plasmin from plasminogen, which in turn simultaneously simulates catabolism and degradation of fibrin and fibrinogen, as some of the plasmin is rapidly inhibited by complex formation with $\alpha_2$-antiplasmin. t-PA, tissue plasminogen activator.

The decrease in PAI-1 and fibrinogen concentrations could also be associated. The present results suggest a link between the fibrinolytic system and plasma fibrinogen. In multivariate analysis, 53% of the change in plasma fibrinogen concentration could be explained by $\alpha_2$-antiplasmin and Lp(a) concentration changes. It is possible that the mobilization of plasmin, as indicated by reductions in PAI-1 activity and $\alpha_2$-antiplasmin concentration, could lead to the degradation of plasma fibrinogen, or enhance fibrinogen catabolism by other means, as it is known that plasmin has affinity for both fibrin and fibrinogen [31] (Fig. 1). Such a hypothesis is supported by a study [8] in which 100 mg nicotinic acid was given intravenously, resulting in enhanced mobilization of plasmin,

incomplete inhibition of plasmin and degradation of fibrinogen. This interpretation is tentative, as we did not undertake any analyses relating to fibrinogen catabolism in the present study.

Increased concentrations of the von Willebrand factor have been shown to correlate with endothelial activity, which sometimes parallels endothelial dysfunction [32]. The increase in von Willebrand factor concentration as a result of treatment with nicotinic acid requires clarification in further studies.

In conclusion, long-term treatment with nicotinic acid in gram doses corrects aberrations in lipoprotein concentrations, stimulates fibrinolysis and decreases plasma concentrations of fibrinogen. These effects may in part explain the beneficial outcome in prospective studies in which nicotinic acid has been used and there has been a decreased incidence of CHD [4,5].

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