Plasma homocysteine and the risk of venous thromboembolism: insights from the FIELD study

Markus Herrmann, Malcolm J Whiting, Anne-Sophie Veillard, Christian Ehnholm, David R Sullivan, Anthony C Keech; for the FIELD study investigators

Department of Clinical Biochemistry, Royal Prince Alfred Hospital, Sydney, Australia (MH, DRS), Flinders Medical Centre, Adelaide, Australia (MJW), National Health and Medical Research Council Clinical Trials Centre, University of Sydney, Sydney, Australia (ASV, ACK), National Public Health Institute, Biomedicum, Finland (CE)

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Correspondence and requests for reprints to:

Professor AC Keech, or Associate Professor Markus Herrman, FIELD trial, c/o NHMRC Clinical Trials Centre, University of Sydney, NSW 2006, Australia. Email: FIELDtrial@ctc.usyd.edu.au; Tel: 61 2 9562 5000; Fax: 61 2 9565 1863

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Abstract

Background

The lipid-lowering effect of fenofibrate is accompanied by a rise in plasma homocysteine, a potential risk factor for venous thromboembolism (VTE). This study investigated the relationship between homocysteine and the risk of VTE in patients treated with fenofibrate.

Methods and results

The relationship between homocysteine and deep-vein thrombosis or pulmonary embolism was investigated in 9522 participants of the 5-year Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial. All subjects received fenofibrate during a 6-week active run-in phase before randomization. A Cox proportional-hazards model was used to assess the effect of homocysteine on risk of venous thromboembolic events.

During active-drug run-in, homocysteine rose on average by 6.5 μmol/L, accompanied by a substantial rise in plasma creatinine (+12%). Fenofibrate-induced changes in homocysteine and creatinine were fully reversible in the placebo group but persisted in the treatment group until reversing at the end of therapy. During follow-up, 1.8% had at least one episode of deep-vein thrombosis or pulmonary embolism: 103 on fenofibrate and 68 on placebo (log-rank P=0.006).

In multivariate analysis, every 5 μmol/L higher baseline homocysteine was associated with 19% higher risk of VTE. Fenofibrate treatment was associated with 52% higher risk, but the change in homocysteine with fenofibrate was not significantly associated with VTE after adjustment for baseline homocysteine.

Conclusions

Hyperhomocysteinemia is prospectively associated with VTE. Fenofibrate may predispose individuals with high pretreatment homocysteine towards VTE. The fenofibrate-induced increase in homocysteine did not, however, explain the risk associated with fenofibrate therapy.

Key words:

Thrombosis; homocysteine; fenofibrate; diabetes mellitus; pulmonary embolism

Abbreviations:

ACCORD	Action to Control Cardiovascular Risk in Diabetes
CVD	cardiovascular disease
FIELD	Fenofibrate Intervention and Event Lowering in Diabetes
GFR	glomerular filtration rate
VTE	venous thromboembolism

Introduction

Lipid-lowering drug therapy is one of the mainstays of primary and secondary prevention of cardiovascular disease [1-3]. Although statins are the most commonly prescribed lipid-lowering agents, they are not the most effective agents for lowering triglycerides [3]. Fibrates, such as fenofibrate, are known to lower triglycerides and, to a lesser extent, cholesterol [4]. Fibrate therapy is of particular relevance in diabetes, where triglycerides are often elevated [5-6]. In the large-scale Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial, long-term fenofibrate treatment reduced triglycerides by 27%, total cholesterol by 11%, and LDL cholesterol by 11% in patients with type 2 diabetes.[7] Although fenofibrate did not significantly reduce the risk of the primary outcome of coronary events, it did significantly reduce cardiovascular events. Furthermore, fenofibrate caused a marked reduction in microvascular events including retinopathy, nephropathy and neuropathy. The beneficial effects of fenofibrate were evident, despite an associated increase in the serum level of homocysteine, which is an established risk factor for CVD [8]. The homocysteine-increasing effect of fenofibrate also raises the question whether fenofibrate treatment increases the likelihood of VTE events, because there is some evidence that homocysteine increases venous thrombembolism.[9-11]

Case–control studies from the 1990s reported a high prevalence of mild to moderate hyperhomocysteinemia in VTE patients.[9 12-15] However, the impact of these studies is limited by their retrospective nature and the small number of cases. Prospective studies have been rare, with a total of 476 cases and 1517 controls in 3 studies.[10] Although they generally confirmed hyperhomocysteinemia as a risk factor, the associations were statistically weak, creating uncertainty about the causality of hyperhomocysteinemia for VTE. The common C677T polymorphism of the

methylenetetrahydrofolate reductase (MTHFR) gene also contributes to elevated homocysteine concentrations, especially when present in the homozygous form, Studies investigating the potential relationship between this polymorphism and venous thromboembolism have been inconsistent. A meta-analysis including 31studies showed a weak association between the MTHFR C677T gene polymorphism and an increased risk of venous thromboembolism [16]. The authors speculated that the relationship between hyperhomocysteinemia and venous thromboembolism is unlikely to be mediated by this gene defect to a substantial degree,

The limited number of prospective studies and their inconclusive outcomes highlight the need for additional prospective data investigating the relationship between serum homocysteine and venous thromboembolism.

Our study investigates the relation between homocysteine and venous thrombolism in the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial, in which patients with type 2 diabetes mellitus were treated with placebo or fenofibrate for an average of 5 years. Since fenofibrate is known to increase homocysteine,[8] we also investigated whether fenofibrate-induced changes in homocysteine modify the risk of future thrombotic events.

Material and Methods

Study design

The FIELD trial was a multinational, double-blind, placebo-controlled trial in 63 centers in Australia, New Zealand, and Finland. The study population consisted of 9795 participants aged 50–75 years who had type 2 diabetes mellitus diagnosed according to WHO criteria and who were not taking lipid-modifying therapy at study

entry. A detailed description of the design of the study has been published.[17] Between February 1998 and November 2000, patients were randomly allocated to once-daily micronised fenofibrate (Laboratoires Fournier, Dijon, France), 200 mg, or placebo. Patients were recruited from hospital clinics and community sources. Exclusion criteria included renal impairment (blood creatinine >130 μmol/L), known chronic liver disease or symptomatic gallbladder disease, and a cardiovascular event within the 3 months before recruitment.

Before randomization all participants entered a standardized run-in phase consisting of a 4-week diet-only period, a 6-week single-blind placebo period, and then a 6-week single-blind active run-in period on the study treatment (Figure 1). This allows determination of the correlation between any long-term clinical effects of treatment and short-term alterations associated with the drug, including changes in circulating homocysteine levels. Homocysteine results and complete medical records were available from 9522 participants at baseline and from 8182 at the end of the active run-in phase.

All patients gave written informed consent. The study protocol was approved by local and national ethics committees and was undertaken in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Blood sampling and measurement of homocysteine

Plasma homocysteine was measured before the 6-week active-treatment run-in (at baseline) and at the end (at randomization). Fasting blood samples were collected into EDTA tubes. Immediately after collection, samples were separated and frozen on site. Frozen samples were transported to one of two central laboratories in Adelaide (Australia) and Helsinki (Finland) where they were aliquoted and stored at -80°C until

analysis. In the Australian laboratory, plasma homocysteine was assayed by high-performance liquid chromatography (HPLC) after reduction and derivatization with a fluorophore. In Finland a commercial immunoassay was used. These methods are known to produce comparable results and were confirmed by the exchange of samples between the two laboratories. In both laboratories assay performance was monitored over time by internal quality-control procedures and participation in local external quality-assurance programs.

Statistical analysis

The main outcome studied was the time from randomization to a deep-vein thrombosis or pulmonary embolism event. Circulating biomarkers were expressed as mean and standard deviation, or median with interquartile range if data were not normally distributed. Calculations of relative change were based on log-transformed data using the ratio of geometric means.

Cumulative hazard curves of events according to treatment group were plotted, and the log-rank test was used to ascertain the effect of fenofibrate treatment on the first venous thromboembolism event. A Cox proportional-hazards model was used to assess the effect of homocysteine on risk of venous thromboembolism events. Hazard ratios (HRs) and 95% confidence intervals are presented. The proportional-hazards assumption was assessed using the Harrell–Lee test[18]. Selection of predictor variables was performed using the backward elimination method. For the purposes of analysis, change in homocysteine was defined as the difference between baseline and end-of-run-in homocysteine levels for each subject in the fenofibrate-randomized group, and zero change was attributed to all subjects in the placebo group (based on the lack of change in average levels in placebo-treated subjects at 1 year).

Since previous studies have speculated about the existence of a homocysteine threshold level above which venous thromboembolism risk starts to rise,[9 19-20] we investigated whether there was evidence for such a threshold in the FIELD cohort. The relationship between quintiles of homocysteine and risk was assessed in an intention-to-treat analysis using an adjusted Cox model. Fractional polynomials, a flexible tool for modelling the relationship between the continuous variable, plasma homocysteine, and venous thromboembolism, were fitted.[21] We considered models of up to 2 degrees.

Analyses were performed using SAS version 9.2, and a two-tailed $P<0.05$ was considered significant.

Results

Baseline characteristics and effects of fenofibrate on circulating biomarkers

Participants in the placebo and fenofibrate groups were well matched for baseline characteristics, such as age, body mass index, smoking habits, blood pressure and duration of diabetes (Table 1). Both groups had a similar history of cardiovascular events. The use of glucose-lowering and cardiovascular medication was as expected for a cohort of people with diabetes and did not differ between the two groups. Baseline homocysteine levels were similar in the placebo group (table 2) and the fenofibrate group. Similarly, other biochemical serum or plasma markers including creatinine, fibrinogen and lipoprotein (Lp) (a) also showed comparable baseline levels.

While placebo treatment had no effect on any relevant biomarker over the short-to-medium term (1 year), fenofibrate induced significant changes in homocysteine and creatinine. During the active run-in phase (before randomization; all participants were treated with fenofibrate for 6 weeks), plasma homocysteine rose on average by 6.5

μmol/L in both groups. The increase in homocysteine was accompanied by a substantial rise in plasma creatinine by 10.0 μmol/L (+12%). A Spearman's correlation analysis showed a significant correlation between the change in homocysteine and the change in creatinine with a correlation coefficient of r=0.38, P <0.001.

Repeat measurements after one and five years of treatment showed that the fenofibrate-induced changes in homocysteine and creatinine were fully reversible in those randomized to placebo after active run-in, but persisted in the treatment group long-term. However, after randomization to long-term fenofibrate, homocysteine stabilized at somewhat lower levels when compared with the initial increase at the end of the 6-week run-in phase. In contrast, creatinine levels remained elevated during the entire follow up period

Description of events

During the follow-up period, 171 patients (1.8% of the entire cohort) had a least one deep-vein thrombosis or pulmonary embolism event (221 events). While most of these patients (76.6 %) had only one event, 23.4 % sustained two or more. In the fenofibrate group 103 patients had an event, compared with 68 patients in the placebo group. (Figure 2; log-rank P=0.006). Overall, deep-vein thrombosis was more frequent than pulmonary embolism. 110 and 85 patients had at least one deep vein thrombosis and pulmonary embolism event, respectively. The HRs comparing the incidence of thrombosis events between fenofibrate-and placebo-treated participants remained relatively constant throughout the trial, with no statistical heterogeneity observed in hazard ratios across arbitrary time periods (0–2 years, 2–4 years, >4 years).

Relationship between homocysteine and the risk of thrombosis events

Before exploring the relationship between homocysteine and thrombosis risk, potential confounders were identified using univariate and multivariate analyses. These analyses identified the following parameters as significant predictors of thrombotic risk: age, BMI, ethnicity, history of hypertension, history of CVD, neuropathy and the albumin/creatinine ratio. Those remaining significant in multivariate analysis were age, ethnicity, history of CVD and BMI. Adjusting for all these potential confounders revealed baseline homocysteine and the use of fenofibrate as significant independent predictors of thrombosis risk (Table 3). Each 5 µmol/L higher plasma homocysteine at baseline was associated with 19% higher risk. Fenofibrate treatment was associated with a 52% higher risk of thrombosis. Fenofibrate appeared more likely to increase thrombotic risk in those with highest baseline homocysteine levels (interaction p=0.04). The change in homocysteine with fenofibrate was not significantly associated with thromboembolism after adjustment for baseline homocysteine level (Table 4).

Is there a homocysteine threshold level above which venous thromboembolism risk increases?

The HRs for venous thromboembolism by quintile of plasma homocysteine level showed no evidence of a threshold effect. In models of the relationship using plasma homocysteine as a continuous variable, none of the models fitted significantly better than the linear model, failing to support the existence of a threshold effect.

Discussion

This study provides evidence that naturally occurring levels of plasma homocysteine are associated with risk of future venous thromboembolism events. A 5 µmol/L difference in plasma homocysteine was associated with a 19% difference in the risk of

thrombotic events. This observation is in keeping with previous case–control studies reporting a high prevalence of hyperhomocysteinemia in venous thromboembolism patients.[9 12-15] Prospective studies are rare, and together comprise a total of only 476 venous thromboembolism cases and 1517 controls.[10] Their results were variable [22-23]. For example, Ridker et al. did not find a higher risk of venous thromboembolism in people with plasma homocysteine levels ≥17.25 μmol/L compared to those with lower levels.[22] Similarly, Tsai et al. found a nonsignificantly higher thromboembolism risk in the highest quintile of serum homocysteine (adjusted odds ratio 1.55; 95% CI, 0.93–2.58).[23] In the present study baseline homocysteine was a significant predictor of thrombotic events in the entire cohort but not in the placebo group suggesting that overall this relationship is not very strong. A meta-analysis, pooling the data from all available prospective studies showed a 27% increase in thrombosis risk (odds ratio 1.27; 95% CI, 1.01–1.59) for every 5 μmol/L higher circulating homocysteine,[10] which is comparable to our result.

The mechanisms underlying the association between homocysteine and venous thromboembolism risk are complex and insufficiently understood. Homocysteine causes oxidative stress and endothelial damage. Furthermore, it activates platelets, promotes local thrombin formation and impairs fibrinolysis [24-25]. The induction of endothelial tissue factor expression and the inhibition of plasminogen activation by tissue plasminogen activator appear to be of pivotal importance [24 26-27]. For example, homocysteine increases the expression of plasminogen activator inhibitor-1 and thrombin-activatable fibrinolysis inhibitor [24 27]. Adverse effects on angiogenesis resulting in angiostasis have also been described [24 26]. It has been suggested that hyperhomocysteinemia potentiates the thrombotic risk in carriers of inherited thrombophilic factors, such as Factor V Leiden or the prothrombin G20210A

mutation [28]. However, existing studies investigating such interactions are contradictory. When compared to normal individuals some studies found a 10- to 50-fold higher venous thromboembolism risk in hyperhomocysteinaemic carriers of a prothrombotic mutation, suggesting a synergistic effect. Other studies were not able to confirm this observation. In fact, the co-existence of the C677T MTHFR homozygous genotype does not increase the thrombotic risk associated with Factor V Leiden or the prothrombin G20210A mutation. As yet, in the FIELD cohort there is no information available about the frequency and distribution of these hereditary prothrombotic conditions.

In our study plasma homocysteine promptly rose during fenofibrate treatment. The concomitant elevation of plasma creatinine suggests a possible alteration in renal metabolism. Previous studies have shown that homocysteine is consistently elevated in patients with renal impairment and glomerular filtration rate (GFR) less than 60 ml/min.[29-31] Although creatinine increased during fenofibrate therapy in FIELD, the albumin/creatinine ratio did not rise suggesting that the excretory renal function was not impaired. In addition, the correlation between the change in homocysteine and the change in creatinine was relatively weak (R=0.38) suggesting that an altered renal homocysteine handling explains only a minor fraction of this effect. When a subset of patients was retested after discontinuation of fenofibrate at the end of the study, homocysteine levels returned to baseline whilst creatinine levels even fell slightly below baseline levels, implying significant reno-protective effects [32]. Therefore, we speculate that the elevation of homocysteine, and possibly creatinine, is due to increased synthesis via PPAR alpha activation, as suggested by Luc et al.[33]

Despite the beneficial effects of fenofibrate on various homoeostatic indices [34] and inflammatory markers [35] the present results suggest fenofibrate use as an independent

risk factor for venous thromboembolism (52% risk increase). Although fenofibrate also induces a substantial rise in plasma homocysteine levels, the lack of an interaction between fenofibrate use and the extent of increase in homocysteine levels does not support this being the sole mechanism of the greater risk associated with its use. In a previous study Lacut et al. also observed an increased risk of venous thromboembolism events in fenofibrate users which was independent of plasma homocysteine (OR1.88; 95% CI 1.29 – 2.74) [36]. An altered lipid handling may at least partly be responsible for this observation. Fenofibrate for example induces the expression of uncoupling proteins (UCP), such as UCP 2 and 3, in liver and skeletal muscle [37]. Furthermore, it affects elongase and desaturase, which are important in fatty acid metabolism [38-39]. This may lead to an altered lipid-profile favouring the formation of clots [40]. In contrast to the present findings, other studies, such as the recently published ACCORD-Lipid trial, have reported fenofibrate to be safe and did not observe increased rates of venous thromboembolism [41-42]. In the context of a relatively weak interaction between baseline homocysteine and fenofibrate this raises the possibility that the increased risk associated with fenofibrate in FIELD may be a chance false-positive finding from among the several hundred types of safety outcomes collected in the FIELD study. Another possibility is that the result is confounded by the greater number of patients in the placebo group commencing statin use during the study. Statin therapy has been shown to have a modest protective effect against venous thromboembolism events,[43] but a multivariate model adjusting for differences in statin use confirmed baseline homocysteine and fenofibrate as independent predictors of future venous thromboembolism. ## The results may reflect the variable interaction of several factors over the duration of the study. In the early phase, the interaction between baseline homocysteine and fenofibrate induced

increase in homocysteine may have predominated, whilst in the later phase, when the fenofibrate induced increase in homocysteine had abated, the higher rate of statin use may have provided relative protection towards the placebo group. The baseline levels of other potential thrombophilic risk factors, such as fibrinogen and Lp(a),[25-2829-32] did not show significant associations with future thrombotic events. This emphasizes the role of baseline homocysteine and fenofibrate treatment as independent risk factors for venous thrombemolism.

Den Heijer et al. suggested that venous thromboembolism risk starts to rise if natural homocysteine levels exceed a threshold of about 17 µmol/L.[9] Despite higher on-study plasma homocysteine levels (approximately 4 µmol/L) and significantly more venous thromboembolism events events (+52%) in the fenofibrate group when compared to the placebo group, all our analyses failed to confirm the existence of such a threshold. The FIELD cohort had relatively low average plasma homocysteine, and only a few participants had elevated plasma homocysteine levels. This may have prevented the detection of a homocysteine threshold above which venous thromboembolism risk starts to rise.

In conclusion, data from the FIELD trial confirm previous findings that suggest that natural homocysteine levels are an independent risk factor for venous thromboembolism events. Unlike the ACCORD Lipid study, in which all participants received statin therapy, the use of fenofibrate in FIELD increased the risk for thrombotic events, especially in patients with a high pre-treatment plasma homocysteine. Although fenofibrate increased homocysteine levels in FIELD, there was no interaction between the extent of homocysteine elevation, fenofibrate use and venous thromboembolism events risk, It remains to be determined whether or not the use of homocysteine-lowering treatments, such as folate and vitamin B12

supplementation,[44] might ameliorate any true fenofibrate-related risk of venous thromboembolism events.

Sources of funding

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Disclosures

None

Table 1: Baseline characteristics of the study population

Characteristics*	Placebo (*n*=4767)	Fenofibrate (*n*=4755)
Anthropometric data		
Male	2972 (62.3%)	2972 (62.5%)
Age at visit 1 (years, mean[SD])	62.2 (6.9)	62.2 (6.9)
Diabetes duration (years, median[IQR])	5 (2–10)	5 (2–10)
Body-mass index(kg/m^2, median[IQR])	29.8 (26.8–33.4)	29.8 (26.8–33.7)
Blood pressure: systolic (mmHg, mean[SD])	140.4 (15.3)	140.4 (15.4)
Blood pressure: diastolic (mmHg, mean[SD])	81.9 (8.5)	82.0 (8.5)
Current smoker	449 (9.4%)	441 (9.3%)
Exsmoker	2432 (51.0%)	2392 (50.3%)
Clinical history		
Prior cardiovascular disease	1031 (21.6%)	1026 (21.6%)
Prior myocardial infarction	247 (5.2%)	217 (4.6%)
Prior stroke	175 (3.7%)	159 (3.3%)
Prior angina	566 (11.9%)	570 (12.0%)
Prior coronary revascularization	162 (3.4%)	184 (3.9%)
History of hypertension	2678 (56.2%)	2700 (56.8%)
History of diabetic retinopathy	396 (8.3%)	388 (8.2%)
History of diabetic neuropathy	661 (13.9%)	672 (14.1%)
History of diabetic nephropathy	130 (2.7%)	139 (2.9%)
Baseline cardiovascular medication		
Warfarin	114 (2.4%)	118 (2.5%)
Antiplatelet alone	1380 (28.9%)	1367 (28.7%)
Neither	3273 (68.7%)	3270 (68.8%)
Baseline blood-glucose-lowering medication		
Diet alone	1283 (26.9%)	1266 (26.6%)
Insulin	645 (13.5%)	646 (13.6%)
Metformin, sulfonylurea, or other oral agent	2839 (59.6%)	2843 (59.8%)

* Normally distributed variables are shown as mean (SD), non-normally distributed as median (25th to 75th interquartile range)

Table 2: Serial measurements of relevant biochemical markers. Results are shown as mean (SD) if distributed normally or median (25th to 75th quartile)

Marker and time points	Placebo (*n*=4767)	Fenofibrate (*n*=4755)
Homocysteine (umol/L)		
Baseline	9.6 (8.0–11.4)	9.5 (7.9–11.6)
End of run-in	15.9 (13.5–19.1)	16.0 (13.5–19.2)
1 year	9.4 (8.0–11.4)	13.4 (11.0–16.4) †
Study end	11.1 (9.3–13.6)	15.1 (12.3–18.7) †
Creatinine (umol/L)		
Baseline	77.3 (15.7)	77.7 (15.9)
End of run-in	87.2 (20.3)	87.8 (20.6)
1 year	76.5 (19.4)	88.7 (22.6) †
Study end	85.3 (26.3)	96.4 (34.0) †
Albumin/creatinine (mg/mmol)		
Baseline	1.10 (0.60–2.90)	1.15 (0.60–3.00)
Study end	1.00 (0.40–3.10)	0.80 (0.40–2.40) †
Fibrinogen (g/L)		
Baseline	3.58 (0.73)	3.60 (0.75)
End of run-in	3.11 (0.74)	3.12 (0.77)
1 year	3.42 (0.90)	2.94 (0.79) †
Study end	4.05 (0.97)	3.45 (0.89) †
Lipoprotein(a) (mg/L)		
Baseline	91 (35–263)	89 (36–258)
End of run-in	96 (39–265)	96 (39–260)
Study end	100 (48–256)	86 (40–217)*
Apolipoprotein A2 (g/L)		
Baseline	0.35 (0.07)	0.35 (0.07)
End of run-in	0.45 (0.10)	0.45 (0.10)
Study end	0.35 (0.08)	0.45 (0.11) †

* *P*<0.05, † P<0.001 for the comparison between treatment groups.

Table 3: Multivariate analysis of predictors of venous thrombosis events

Predictor	Hazard ratio (95% CI)	*P*
Caucasian	3.09 (1.15–8.35)	0.03
Age (years)	1.03 (1.01–1.06)	0.008
Prior cardiovascular disease	1.45 (1.04–2.02)	0.03
Body-mass index (kg/m^2)	1.07 (1.05–1.10)	<0.001
Homocysteine at baseline (per 5 µmol/L)	1.19 (1.06–1.32)	0.002
Fenofibrate	1.52 (1.12–2.06)	0.008

Table 4: Effect of change in homocysteine between baseline and end of run-in risk of venous thrombosis

Model and variables	Hazard ratio (95% CI)	*P*
Difference in homocysteine between baseline and end of run-in (per 5 µmol/L)	1.05 (0.83–1.33)	0.71
Fenofibrate	1.40 (0.87–2.26)	0.17
Homocysteine at baseline (per 5 µmol/L)	1.20 (1.04–1.40)	0.01

* Model was adjusted for age, ethnicity (Caucasian vs. other), body-mass index at baseline and prior cardiovascular disease.

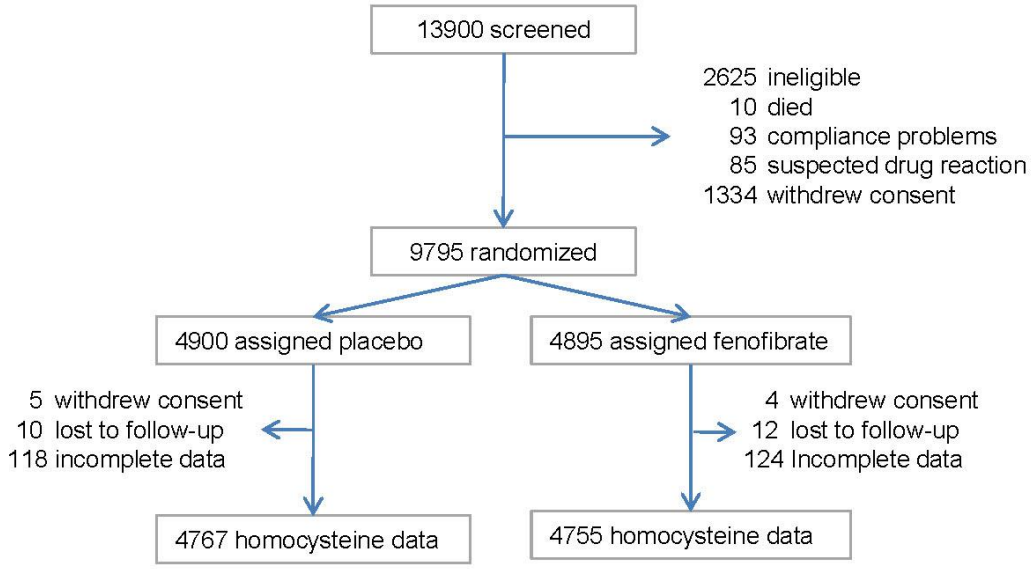
Figure legends

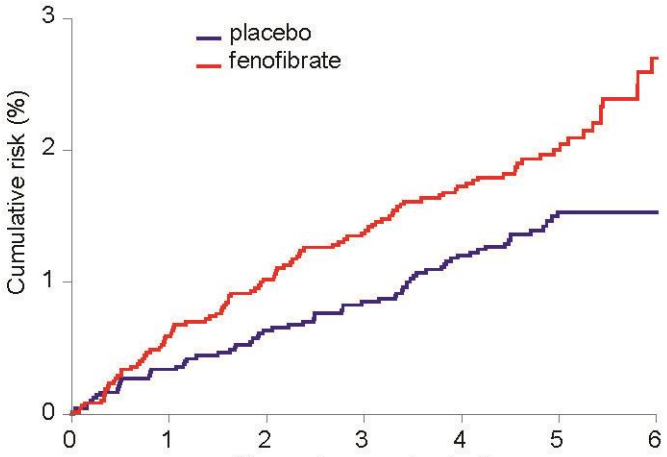
Figure 1

Flow chart illustrating the design of the FIELD study.

Figure 2

Hazard plot showing the cumulative risk of the time to first deep-vein thrombosis or pulmonary embolism, by treatment group.


13900 screened
2625 ineligible
10 died
93 compliance problems
85 suspected drug reaction
1334 withdrew consent
9795 randomized
4900 assigned placebo
4895 assigned fenofibrate
5 withdrew consent
10 lost to follow-up
118 incomplete data
4 withdrew consent
12 lost to follow-up
124 Incomplete data
4767 homocysteine data
4755 homocysteine data



Number at risk							
Placebo	4767	4718	4657	4596	4515	2518	803
Fenofibrate	4755	4700	4622	4543	4442	2494	793

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