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# Hemostatic effects of bezafibrate and ω-3 fatty acids in isolated hypertriglyceridemic patients

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**Abstract:**
This study aimed to compare the effects of ω-3 fatty acids and fibrate treatment on plasma levels and activities of hemostatic risk factors on glucose and lipid metabolism in subjects with isolated hypertriglyceridemia. Seventy-three subjects with elevated triglyceride levels were allocated into one of the following treatment options: bezafibrate (200 mg twice daily), ω-3 fatty acids (1 g twice daily) or placebo. Plasma lipids, glucose homeostasis markers (fasting and 2-h post-glucose load plasma glucose levels and HOMA), as well as plasma levels/activities of fibrinogen, factor VII and PAI-1 were determined at baseline, on the day of randomization, and after 4 and 12 weeks of the treatment. Not only did bezafibrate improve plasma lipids, but it also increased glucose sensitivity and tended to reduce post-glucose loads of plasma glucose. Except for the reduction in plasma triglycerides, ω-3 fatty acids produced no effect on the lipid profile and insulin sensitivity. Both treatment options reduced, to similar extents, plasma levels of fibrinogen and PAI-1 and factor VII coagulant activity. Our study indicates that, although fibrates exhibit more-pronounced metabolic effects than do ω-3 fatty acids, both these treatment options are equipotent in producing a complex beneficial effect on hemostasis in isolated hypertriglyceridemic subjects.

**Key words:**
fenofibrate, ω-3 fatty acids, hypertriglyceridemia, fibrinogen, factor VII, plasminogen activator inhibitor-1

**Abbreviations:** BIP – Bezafibrate Intervention Prevention Study, EPA – eicosapentaenoic acid, GISSI – Gruppo Italiano per lo Studio della Sopravvivenza Nell'Infarto Miocardico, HDL – high-density lipoprotein, INR – International Normalized Ratio, JELIS – Japan EPA Lipid Intervention Study, LDL – low-density lipoprotein, OGTT – oral glucose tolerance test, PAI-1 – plasminogen activator inhibitor-1, PPARα – peroxisome proliferator-activated receptor-α, VA-HIT – Veterans Affairs High-Density Lipoprotein Intervention Trial

## Introduction

The rupture of an atherosclerotic plaque followed by thrombus formation is the major cause of atherosclerosis-related cardiovascular and cerebrovascular events [25]. Disturbances of coagulation and fibrinolysis, which are two separate but reciprocally linked enzyme cascades that regulate the formation and breakdown of fibrin, contribute to the development and progression of atherosclerosis [24]. Elevated plasma levels/activities of fibrinogen, plasminogen activator inhibitor-1 (PAI-1), factor VII, factor VIII, von Willebrand factor, soluble thrombomodulin, and tissue plasminogen activator are considered important cardiovascular risk factors that determine the risk of accelerated progression of atherosclerosis and the development of its complications [16, 24].

The results of several clinical studies have revealed that hypertriglyceridemia markedly increases cardio-

vascular morbidity and mortality, which is observed in middle-aged and elderly men [14], subjects who have undergone diagnostic coronary arteriography [27] and patients with coronary artery disease [2]. Each 1 mmol/l increase in triglyceride levels increases the incidence of cardiovascular events by 76% in women and 32% in men [1]. Because hypertriglyceridemia has been found to be associated with abnormal hemostasis [36], disturbed coagulation and fibrinolysis may be partially responsible for increased vascular risk and premature development of coronary artery disease.

Peroxisome proliferator-activated receptor-α (PPAR-α) activators, or fibrates, and ω-3 fatty acids are clinical options in the treatment of hypetriglyceridemia [3, 36]. There are some arguments that both fibrates and ω-3 fatty acids improve hemostasis. For PPAR-α activators, this effect was observed in patients with impaired glucose tolerance [29], impaired fasting glucose [21], overt diabetes [31], mixed dyslipidemia [28] and metabolic syndrome [19], but not in healthy subjects [22]. In turn, ω-3 fatty acid-induced improvement in hemostasis was found in patients with type 2 diabetes [15], mixed dyslipidemia [8], coronary artery disease [26] and 45–64-year-old adults [33], but not in moderately hyperlipidemic subjects [10] and healthy male subjects [5].

To the best of our knowledge, only one small study compared the strength of hemostatic effects of fibrates and ω-3 fatty acids. Otto et al. [30] observed that fenofibrate, but not ω-3 fatty acids, decreased fibrinogen levels as well as plasma and blood viscosity in 8 subjects with familial dysbetalipoproteinemia**.** Therefore, in this study we determined the effect of bezafibrate and ω-3 fatty acids on plasma levels/activities of fibrinogen, factor VII and PAI-1 in isolated hypertriglyceridemia. The assessed markers are major hemostatic risk factors for coronary artery disease, and even small differences in their plasma levels/activity are associated with different cardiovascular risk [16, 24].

## Materials and Methods

### Patients

Subjects with recently diagnosed and previously untreated isolated hypertriglyceridemia were eligible for the study if they met the following criteria: triglyceride levels between 200 and 500 mg/dl, total cholesterol less than 200 mg/dl and LDL cholesterol less than 130 mg/dl. The exclusion criteria were as follows: age > 70 or < 30 years, severe hypertriglyceridemia (triglycerides above 500 mg/dl), diabetes mellitus, any acute and chronic inflammatory processes, congestive heart failure, unstable coronary artery disease, myocardial infarction or stroke within 6 months preceding the study, moderate or severe arterial hypertension (ESC/ESH grade 2 or 3), impaired renal or hepatic function, concomitant treatment with other agents known either to affect plasma lipid levels or to interact with fibrates or ω-3 fatty acids, treatment with drugs that may affect inflammatory processes in the vascular wall (including glucocorticosteroids, nonsteroidal anti-inflammatory drugs, calcium channel blockers, angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers) within 3 months preceding the study, ongoing hormonal replacement therapy or oral contraception, and inadequate patient compliance.

All participants provided written informed consent prior to participation in this study. The regional ethical committee approved the study protocol, and the study was conducted in accordance with the Declaration of Helsinki Principles.

### Study design

All enrolled patients were informed regarding the principles of a healthy diet and instructed to stop smoking and to cease alcohol consumption, and they were encouraged to participate in either moderately or vigorously intensive physical activity at least five days of the week. The average dietary adherence was assessed by the food frequency questionnaire and by analysis of 3 days' eating diaries by validated methods at every visit. After 4 weeks, the subjects were randomized into one of the three treatment groups and were treated with bezafibrate (200 mg twice daily, n = 25), ω-3 fatty acids (2 g daily in two divided doses, n = 24) or placebo (twice daily, n = 24), respectively. ω-3 fatty acids were administered in the form of capsules containing high concentrations of highly purified ω-3 fatty acid ethyl esters (1 g in each capsule), mainly consisting of eicosapentaenoic acid (EPA) (465 mg) and docosahexaenoic acid (375 mg) (Omacor, Solvay Pharmaceuticals). The placebo was composed of a starch preparation with the same weight as that of

bezafibrate and ω-3 fatty acid capsules. Bezafibrate and ω-3 fatty acids were administered for 12 weeks without any changes in dosage throughout the study. During the entire study, all included patients complied with lifestyle modification. Compliance (assessed by tablet counts) and the safety of the treatment were determined twice monthly.

### Laboratory assays

Plasma lipids, fasting plasma glucose, 2-h postchallenge plasma glucose (in the 75-g oral glucose tolerance test), plasma insulin, and hemostatic markers were assessed at baseline, on the day of randomization (after completing the 4-week adaptation period), and after 4 and 12 weeks of hypolipidemic treatment. Venous blood samples were taken 12 h after the last meal from the antecubital vein in a temperature-controlled room (24–25°C) and collected into tubes containing ethylenediaminetetraacetic acid. To avoid possible circadian fluctuations in plasma fibrinogen and PAI-1 levels [24] samples were drawn during constant daily hours (between 8.00 and 9.00 a.m.). All measurements were performed in duplicate.

The plasma levels of total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides were assessed colorimetrically using commercially available kits purchased from bioMerieux (Marcy l'Etoile, France). LDL levels were assessed directly. Plasma glucose were measured using a glucose oxidase method (Beckman, Palo Alto, CA, USA), and insulin levels were measured with a radioimmunoassay using reagents obtained from Linco Research Inc. (St. Charles, MO, USA). The homeostatic model assessment (HOMA) was calculated as fasting insulin concentration (μU/ml) × fasting glucose concentration (mmol/l)/22.5. The International Normalized Ratio (INR) and the partial thromboplastin time were assessed by an automated blood coagulation analyzer SYSMEX CA-540 using reagents purchased from Dade Behring (Germany). INR was measured using Innovin thromboplastin with an international sensitivity analysis of 1.03 for the entire study period. Fibrinogen and factor VII were determined with a semi-automated blood coagulation analyzer OPTION 2 Plus using reagents obtained from bioMerieux (Marcy l'Etoile, France). Fibrinogen was measured with the Clauss method, whereas factor VII coagulant activity was determined by a one-step method using factor VII-deficient plasma. PAI-1 antigen levels were assessed with an enzyme-linked immunosorbent assay (Asserachrom, Diagnostica Stago, France) [20, 23]. The intra- and inter-assay coefficients of variation in our laboratory were as follows: fibrinogen – 2.4% and 3.7%, factor VII – 3.3% and 4.2%, and PAI-1 – 5.0% and 8.6%.

### Statistical analyses

Statistical analyses were performed using GraphPad Prism 2.01 software (GPA-26576-117) and Statistica 6.1 (axxp308a903804ar). The groups were compared using one-way ANOVA followed by Bonferroni's *post-hoc* test (lipid profile and plasma glucose) or using the Kruskall-Wallis test followed by the Mann-Whitney U test (HOMA, fibrinogen, factor VII and PAI-1). The Student's paired *t*-test (lipid profile and plasma glucose) or the Wilcoxon test (HOMA, fibrinogen, factor VII and PAI-1) were applied to compare pre-, inter- and post-therapy data within the same treatment group. Correlations between metabolic variables and the markers of hemostasis were assessed using Kendall's tau test. Values of $p < 0.05$ were considered statistically significant.

## Results

### Baseline characteristics of patients

At study entry, there was no difference between the treatment groups in terms of sex, weight, age, medical background, clinical characteristics and safety parameters (Tab. 1).

### Adverse effects

One subject randomized to ω-3 fatty acids was withdrawn from the study due to nausea and vomiting. Another two patients, one treated with bezafibrate and one receiving placebo, dropped out because of poor compliance with the study protocol. One subject treated with placebo declined further participation in the study because of dizziness. No serious adverse events were observed in the remaining patients throughout the study. All safety parameters remained within normal limits. Sixty-nine subjects completed the study.

**Tab. 1.** Baseline characteristics of the patients[1]

| | Placebo | ω-3 fatty acids | Bezafibrate |
|---|---|---|---|
| Number of patients | 22 | 23 | 24 |
| Age (years) | 52.3 ± 3.1 | 53.4 ± 3.2 | 52.8 ± 2.9 |
| Women (%) | 36.4 | 34.8 | 37.5 |
| Body mass index (kg/m$^2$) | 28.2 ± 2.1 | 28.4 ± 2.3 | 28.6 ± 2.4 |
| Smokers (%) | 40.9 | 34.7 | 33.3 |
| Mild hypertension (%) | 27.3 | 30.4 | 29.2 |
| Stable coronary artery disease (%) | 31.8 | 26.1 | 25.0 |
| Carotid artery atherosclerosis (%) | 18.2 | 17.4 | 16.7 |
| Total cholesterol (mmol/l) | 4.42 ± 0.17 | 4.44 ± 0.17 | 4.47 ± 0.15 |
| LDL-cholesterol (mmol/l) | 2.67 ± 0.10 | 2.65 ± 0.12 | 2.69 ± 0.14 |
| HDL-cholesterol (mmol/l) | 0.94 ± 0.04 | 0.93 ± 0.04 | 0.92 ± 0.05 |
| Triglycerides (mmol/l) | 4.20 ± 0.46 | 4.29 ± 0.52 | 4.25 ± 0.41 |
| Fasting glucose (mmol/l) | 5.33 ± 0.16 | 5.30 ± 0.13 | 5.26 ± 0.17 |
| Two-hour post-glucose load plasma glucose (mmol/l) | 7.54 ± 0.36 | 7.58 ± 0.38 | 7.69 ± 0.39 |
| HOMA ratio | 3.3 ± 0.4 | 3.7 ± 0.4 | 3.6 ± 0.4 |
| INR | 0.83 ± 0.05 | 0.87 ± 0.05 | 0.82 ± 0.06 |
| Partial thromboplastin time (s) | 33.7 ± 2.0 | 33.5 ± 1.2 | 33.4 ± 1.0 |
| Fibrinogen (g/l) | 3.8 ± 0.3 | 4.0 ± 0.4 | 3.7 ± 0.2 |
| Factor VII activity (%) | 155.1 ± 9.3 | 157.2 ± 8.0 | 158.4 ± 7.6 |
| PAI-1 antigen (ng/ml) | 129.2 ± 5.3 | 134.3 ± 6.2 | 136.6 ± 7.0 |

Data are represented as the mean values ± SD. [1]Only data for the 69 individuals who completed the study were included in the final analyses

### Non-pharmacological treatment

No changes in plasma lipids, plasma glucose, insulin sensitivity markers and hemostasis were observed after a 4-week run-in period of non-pharmacological treatment.

### Placebo

Placebo administered for 12 weeks produced no effect on plasma lipids, fasting and 2-h post-challenge plasma glucose, HOMA, INR, the partial thromboplastin time, plasma fibrinogen and PAI-1 levels or factor VII coagulant activity (Tab. 2).

### Bezafibrate

Bezafibrate treatment reduced plasma levels of triglycerides, total and LDL cholesterol, and increased HDL-cholesterol (Tab. 2). The drug also decreased HOMA and insignificantly reduced post-glucose load plasma glucose levels.

After 4 and 12 weeks of therapy, bezafibrate decreased fibrinogen by 21.1% ($p < 0.05$) and 23.7% ($p < 0.05$), PAI-1 antigen by 31.8% ($p < 0.001$) and 55.1% ($p < 0.001$) and factor VII coagulant activity by 22.0% ($p < 0.05$) and 35.4% ($p < 0.001$) (Tab. 3). It did not produce any significant effect on INR and the partial thromboplastin time after 4 weeks of treat-

**Tab. 2.** The effects of bezafibrate and ω-3 fatty acids on the lipid profile, plasma glucose and insulin sensitivity of patients with isolated hypertriglyceridemia[1]

| | Placebo | ω-3 fatty acids | Bezafibrate |
|---|---|---|---|
| Total cholesterol (mmol/l) | | | |
| Baseline | 4.42 ± 0.17 | 4.44 ± 0.17 | 4.47 ± 0.15 |
| The day of randomization | 4.35 ± 0.15 | 4.36 ± 0.14 | 4.44 ± 0.18 |
| After 4 weeks of treatment | 4.37 ± 0.28 (+0.4) | 4.42 ± 0.13 (+1.3) | 3.80 ± 0.13 (–14.4)#*^ |
| After 12 weeks of treatment | 4.41 ± 0.18 (+1.3) | 4.46 ± 0.18 (+2.2) | 3.74 ± 0.13 (–15.8)#*^ |
| LDL cholesterol (mmol/l) | | | |
| Baseline | 2.67 ± 0.10 | 2.65 ± 0.12 | 2.69 ± 0.14 |
| The day of randomization | 2.64 ± 0.12 | 2.62 ± 0.13 | 2.66 ± 0.11 |
| After 4 weeks of treatment | 2.69 ± 0.13 (+1.8) | 2.70 ± 0.10 (+3.0) | 2.33 ± 0.08 (–12.2)#*^ |
| After 12 weeks of treatment | 2.70 ± 0.13 (+2.2) | 2.74 ± 0.11 (+4.6) | 2.32 ± 0.09 (–12.6)#*^ |
| HDL cholesterol (mmol/l) | | | |
| Baseline | 0.94 ± 0.04 | 0.93 ± 0.04 | 0.92 ± 0.05 |
| The day of randomization | 0.95 ± 0.04 | 0.94 ± 0.04 | 0.95 ± 0.05 |
| After 4 weeks of treatment | 0.95 ± 0.05 (0.0) | 1.00 ± 0.05 (+5.7) | 1.15 ± 0.05 (+21.6)##**^ |
| After 12 weeks of treatment | 0.94 ± 0.05 (–1.1) | 1.01 ± 0.05 (+7.1) | 1.20 ± 0.04 (+26.2)###***^^ |
| Triglycerides (mmol/l) | | | |
| Baseline | 4.20 ± 0.46 | 4.29 ± 0.52 | 4.25 ± 0.41 |
| The day of randomization | 3.92 ± 0.34 | 3.98 ± 0.46 | 4.00 ± 0.35 |
| After 4 weeks of treatment | 3.71 ± 0.45 (–5.4) | 3.03 ± 0.34 (–23.6)# | 2.53 ± 0.34 (–36.7)###*** |
| After 12 weeks of treatment | 3.70 ± 0.51 (–5.8) | 2.69 ± 0.45 (–32.4)###** | 2.38 ± 0.30 (–40.5)###*** |
| Fasting glucose (mmol/l) | | | |
| Baseline | 5.33 ± 0.16 | 5.30 ± 0.13 | 5.26 ± 0.17 |
| The day of randomization | 5.22 ± 0.22 | 5.19 ± 0.16 | 5.23 ± 0.18 |
| After 4 weeks of treatment | 5.17 ± 0.21 (–0.9) | 5.25 ± 0.19 (+1.1) | 5.10 ± 0.22 (–2.5) |
| After 12 weeks of treatment | 5.18 ± 0.21 (–0.6) | 5.36 ± 0.23 (+3.1) | 4.99 ± 0.19 (–4.7) |
| 2-h post-glucose load plasma glucose (mmol/l) | | | |
| Baseline | 7.54 ± 0.36 | 7.58 ± 0.38 | 7.69 ± 0.39 |
| The day of randomization | 7.48 ± 0.38 | 7.48 ± 0.31 | 7.66 ± 0.33 |
| After 4 weeks of treatment | 7.40 ± 0.33 (–1.0) | 7.72 ± 0.31 (+3.2) | 7.15 ± 0.29 (–6.6) |
| After 12 weeks of treatment | 7.33 ± 0.34 (–2.0) | 7.79 ± 0.37 (+4.1) | 6.90 ± 0.28 (–9.9)^ |
| HOMA ratio | | | |
| Baseline | 3.3 ± 0.4 | 3.7 ± 0.4 | 3.6 ± 0.4 |
| The day of randomization | 3.2 ± 0.3 | 3.5 ± 0.4 | 3.4 ± 0.3 |
| After 4 weeks of treatment | 3.2 ± 0.3 (–0.1) | 3.7 ± 0.4 (+5.7) | 2.7 ± 0.2 (–20.6)#^^ |
| After 12 weeks of treatment | 3.1 ± 0.4 (–3.1) | 3.9 ± 0.5 (+11.4) | 1.9 ± 0.2 (–44.1)###***^^^$$ |

Data are represented by the mean ± SD. Values in parentheses represent percent changes from values on the day of randomization. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ *vs.* the day of randomization. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* placebo-treated patients. ^ $p < 0.05$, ^^ $p < 0.01$, ^^^ $p < 0.001$ *vs.* omega-3 fatty acid-treated patients. $$ $p < 0.01$ *vs.* respective value after 4 weeks of treatment. [1]Only data for the 69 individuals who completed the study were included in the final analyses

ment, but at the end of the study protocol it increased INR by 34.9% ($p < 0.01$) and prolonged the partial thromboplastin time by 20.7% ($p < 0.05$).

The effect of bezafibrate on HOMA, factor VII and PAI-1 was more pronounced at the end of the study than after 4 weeks of treatment.

### ω-3 Fatty acids

ω-3 Fatty acids reduced plasma triglycerides but did not affect the remaining lipid fractions as well as did insulin sensitivity markers (Tab. 2).

After 4 weeks of treatment, plasma fibrinogen levels, PAI-1 antigen, and factor VII activity decreased by 19.5% ($p < 0.05$), 30.2% ($p < 0.001$), and 20.6% ($p < 0.05$), respectively (Tab. 3). When given for 12 weeks, ω-3 fatty acids reduced fibrinogen by 24.4% ($p < 0.05$), PAI-1 by 52.3% ($p < 0.001$), and factor VII by 34.5% ($p < 0.001$). Twelve weeks of ω-3 fatty acid treatment increased INR by 30.5% ($p < 0.01$) and prolonged the partial thromboplastin time by 19.9% ($p < 0.05$).

The effect of ω-3 fatty acids on factor VII coagulant activity and PAI-1 antigen was stronger after 12 weeks of treatment than after 4 weeks of treatment.

**Tab. 3.** The effect of bezafibrate and omega-3 fatty acids on hemostasis in patients with isolated hypertriglyceridemia[1]

| | Placebo | ω-3 fatty acids | Bezafibrate |
|---|---|---|---|
| INR | | | |
| Baseline | 0.83 ± 0.05 | 0.87 ± 0.05 | 0.81 ± 0.06 |
| The day of randomization | 0.81 ± 0.06 | 0.83 ± 0.04 | 0.82 ± 0.05 |
| After 4 weeks of treatment | 0.84 ± 0.04 | 0.96 ± 0.06 | 0.95 ± 0.05 |
| After 12 weeks of treatment | 0.85 ± 0.05 | 1.12 ± 0.09$^{\#\#*}$ | 1.07 ± 0.08$^{\#\#*}$ |
| Partial thromboplastin time (s) | | | |
| Baseline | 33.7 ± 2.0 | 33.5 ± 1.2 | 33.4 ± 1.0 |
| The day of randomization | 33.2 ± 1.6 | 33.2 ± 1.6 | 32.9 ± 0.6 |
| After 4 weeks of treatment | 34.1 ± 1.8 | 37.2 ± 1.5 | 36.7 ± 0.8 |
| After 12 weeks of treatment | 33.4 ± 1.5 | 39.8 ± 1.3$^{\#*}$ | 39.7 ± 0.6$^{\#*}$ |
| Fibrinogen (g/l) | | | |
| Baseline | 3.8 ± 0.3 | 4.0 ± 0.4 | 3.7 ± 0.2 |
| The day of randomization | 3.9 ± 0.3 | 4.1 ± 0.4 | 3.8 ± 0.3 |
| After 4 weeks of treatment | 4.0 ± 0.4 | 3.3 ± 0.3$^{\#}$ | 3.0 ± 0.2$^{\#*}$ |
| After 12 weeks of treatment | 3.8 ± 0.2 | 3.1 ± 0.2$^{\#*}$ | 2.9 ± 0.2$^{\#*}$ |
| Factor VII activity (%) | | | |
| Baseline | 155.1 ± 9.3 | 157.2 ± 8.0 | 158.4 ± 7.6 |
| The day of randomization | 152.6 ± 4.8 | 156.3 ± 7.4 | 157.0 ± 8.2 |
| After 4 weeks of treatment | 157.8 ± 5.1 | 124.1 ± 6.8$^{\#*}$ | 122.4 ± 6.4$^{\#*}$ |
| After 12 weeks of treatment | 159.0 ± 5.6 | 103.0 ± 4.6$^{\#\#\#***\wedge}$ | 101.4 ± 4.8$^{\#\#\#***\wedge}$ |
| PAI-1 antigen (ng/ml) | | | |
| Baseline | 129.2 ± 5.3 | 134.3 ± 6.2 | 136.6 ± 7.0 |
| The day of randomization | 131.0 ± 6.2 | 132.2 ± 5.8 | 134.1 ± 6.9 |
| After 4 weeks of treatment | 125.4 ± 6.8 | 92.2 ± 6.7$^{\#\#\#**}$ | 91.5 ± 8.4$^{\#\#\#**}$ |
| After 12 weeks of treatment | 128.7 ± 7.4 | 63.1 ± 5.9$^{\#\#\#***\wedge\wedge\wedge}$ | 60.2 ± 5.9$^{\#\#\#***\wedge\wedge\wedge}$ |

Data are represented as the mean values ± SD. $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$, $^{\#\#\#}$ $p < 0.001$ *vs.* the day of randomization. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* control group. ^ $p < 0.05$ ^^^ $p < 0.001$ *vs.* values after 4 weeks of treatment. [1]Only data for the 69 individuals who completed the study were included in the final analyses

### Comparisons between bezafibrate and ω-3 fatty acids

Bezafibrate treatment was superior to ω-3 fatty acids in reducing total cholesterol, LDL cholesterol and HOMA and in increasing HDL cholesterol (Tab. 2). Both these treatment options were equipotent when it comes to affecting hemostasis (Tab. 3).

### Correlations

No correlations between baseline values were observed. Bezafibrate, but not ω-3 fatty acid, action on plasma fibrinogen, factor VII and PAI-1 correlated with its effect on HOMA (r values between 0.48 and 0.58, $p < 0.001$). None of the treatment groups showed any correlation between the changes in hemostasis and insulin sensitivity and the improvement in the lipid profile.

## Discussion

Our study is the first to show that bezafibrate and ω-3 fatty acids are equipotent when it comes to producing a multi-directional favorable effect on coagulation and fibrinolysis. Taking into account the postulated strong association between the increased plasma levels/activity of fibrinogen, factor VII and PAI-1 and the risk of coronary artery disease [16, 24], this effect may in part explain the clinical benefits of these two treatment options in the prevention and treatment of vascular events observed in clinical trials [7, 9, 13, 35]. The beneficial effect on hemostasis observed in our study is also in line with the results of the Bezafibrate Intervention Prevention (BIP) study. In this trial, which involved participants with coronary artery disease, bezafibrate reduced the risk of myocardial infarction and sudden death, particularly in individuals with triglyceride levels exceeding 200 mg/dL, whereas in those with plasma triglycerides below this threshold value, the impact of bezafibrate was much more limited [12].

Hemostatic effects of bezafibrate and ω-3 fatty acids were unrelated to the improvement in the lipid profile, indicating that treatment-induced changes in fibrinogen, factor VII and PAI-1 represent pleiotropic effects of these agents. Interestingly, the effects of bezafibrate on hemostasis correlated with the degree of improvement in insulin sensitivity. This observation is in line with the results of the Veterans Affairs High-Density Lipoprotein Intervention Trial (VA-HIT) [32]. In this trial, a gemfibrozil-induced reduction in cardiovascular events and mortality was particularly pronounced in subjects suffering from type 2 diabetes and in other individuals with hyperinsulinemia, and only less than 25% of the benefits of gemfibrozil treatment could be explained by the impact of this agent on lipid profile. Because the correlation between the effect of bezafibrate on hemostasis and HOMA was moderate, it seems that other mechanisms may also be implicated in the action of PPAR-α activators on hemostasis in isolated hypertriglyceridemic subjects. Such a role may be played by free fatty acids, which are endogenous ligands for PPAR-α receptors [17] that, upon fibrate treatment, are decreased in the plasma [29]. Moreover, the favorable impact of bezafibrate on coagulation and fibrinolysis may be indirect *via* reducing oxidized LDLs. PPAR-α activators were found to reduce plasma levels of these particles, which were found to enhance tissue factor expression on macrophages and are able to interfere with endothelial thrombomodulin expression and reduce fibrinolysis [18].

Interestingly, in the GISSI-Prevenzione trial, an EPA/docosahexaenoic acid treatment-induced reduction in the primary end point-including death from any cause, nonfatal myocardial infarction and nonfatal stroke was observed in myocardial infarction survivors after only 12 weeks of treatment, whereas the maximal decrease in the incidence of sudden death was observed at 9 months of treatment (in the case of statins, a 1- to 2-year treatment period is required to reach their maximal effect) [11, 13]. This may suggest that extra-lipid rather than lipid-lowering effects of ω-3 fatty acids may be responsible for clinical benefits of ω-3 fatty acids. In our unpublished observations, ω-3 fatty acid treatment did not affect monocyte cytokine release and resulted in only small changes in lymphocyte secretory function, despite a strong anti-inflammatory action exhibited by PPAR-α activator treatment [Krysiak et al., submitted]. This indicates that the early benefits of ω-3 fatty acid treatment are likely to be a consequence of ω-3 fatty-acid-induced pleiotropic effects, rather than of anti-inflammatory effects. Apart from postulated anti-arrhythmic properties [4] and the postprandial stress-reducing effect [34], lipid-independent actions may, as our study sug-

gests, include improvements in coagulation and fibrinolysis. Because PPARα activator treatment is associated with a risk of side effects, the most common of which are gastrointestinal disturbances (nausea and diarrhea), headaches, anxiety, fatigue and skeletal muscle-related events [6], ω-3 fatty acids may be considered an alternative treatment option in subjects in whom fibrate therapy is either contraindicated (as a result of severe liver dysfunction, myopathy, or pre-existing gallstone disease, for example) or results in adverse effects.

For factor VII and PAI-1, the effect of both hypolipidemic treatment options was more prominent at the end of the study protocol than after 4 weeks of treatment. This finding may indicate that the full effect of bezafibrate and ω-3 fatty acids on coagulation and fibrinolysis requires at least several months to develop. This may explain why in the BIP, the reduction in the combined end point of cardiac death and nonfatal myocardial infarction was more evident when the follow-up period was extended [12].

Our study has shown that bezafibrate was superior to ω-3 fatty acids in affecting lipid profile and glucose homeostasis. In opposition to the latter treatment option, bezafibrate administration resulted in a reduction in total and LDL cholesterol and an increase in HDL cholesterol. Moreover, only bezafibrate administration reduced insulin resistance and slightly decreased post-glucose load plasma glucose levels. This may suggest that subjects with the concomitant presence of hypertriglyceridemia and glucose homeostasis abnormalities may be better candidates for fibrates than for ω-3 fatty acids. Interestingly, we have recently found that pleiotropic effects of PPAR-α activators in subjects with the metabolic syndrome were stronger if subjects had concomitant prediabetes [19]. On the other hand, because the global hemostatic effect was similar for both treatment options, the possibility that the subgroup of hypertriglyceridemic patients with normal glucose sensitivity responded better to administration of ω-3 fatty acids cannot be excluded.

This study has some limitations. The first one is a relatively small population of patients. Second, because of a short period of treatment, the question of whether long-term treatment more potently affects coagulation and fibrinolysis requires further studies. Finally, our patients were treated with either bezafibrate or ω-3 fatty acids. Therefore, it remains unknown whether their combined administration is associated with any additional benefits, compared with monotherapy.

In summary, our study has revealed that bezafibrate and ω-3 fatty acids produce a similar effect on hemostasis, although only the former therapy is associated with metabolic benefits. Bezafibrate- and ω-3 acid-induced restoration of normal hemostasis may contribute to the clinical effectiveness of these therapies in the prevention and treatment of atherosclerosis and its complications.

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