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Comparison of the effects of hypolipidaemic treatment on monocyte proinflammatory cytokine release in men and women with type 2 diabetes and atherogenic dyslipidaemia

Porównanie wpływu leczenia hipolipemicznego na wydzielanie cytokin prozapalnych u kobiet i mężczyzn z cukrzycą typu 2 i dyslipidemią aterogenną

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Abstract

Introduction: Statins and fibrates reduce monocyte release of proinflammatory cytokines, but it remains unknown whether this effect is sex dependent.

Material and methods: We retrospectively analysed age-, weight-, and lipid-matched populations of type 2 diabetic patients of both sexes, who, because of atherogenic dyslipidaemia, were treated with simvastatin (40 mg daily), fenofibrate (200 mg daily), or simvastatin plus fenofibrate. Monocyte release of tumour necrosis factor α (TNF-α), inteleukin-1β, interleukin-6, and monocyte chemoattractant protein-1 (MCP-1), as well as circulating levels of high-sensitivity C-reactive protein (hsCRP) and free fatty acids (FFA) were assessed separately for men and women before and after 12 weeks of treatment.

Results: Baseline monocyte release of TNF-α, interleukin-1β, interleukin-6, and MCP-1, as well as plasma hsCRP and FFA levels were comparable in both sexes. Simvastatin, fenofibrate, and simvastatin/fenofibrate combination therapy reduced monocyte release of TNF-α, inteleukin-1β, interleukin-6, and MCP-1, with no difference between the treatment groups. The impact of simvastatin and fenofibrate administered alone on monocyte cytokine release and systemic inflammation did not differ between the men and women. The effect of simvastatin/fenofibrate combination therapy on monocyte release of interleukin-6 and MCP-1 was more pronounced in the male population. The impact of simvastatin administered together with fenofibrate on TNF-α, interleukin-1β, and hsCRP was also stronger in the men than in the women, but the difference did not reach the level of significance.

Conclusions: The obtained results suggest that sex differences determine the strength of the monocyte-suppressing effect of simvastatin/ /fenofibrate combination therapy in type 2 diabetic patients with atherogenic dyslipidaemia. **(Endokrynol Pol 2015; 66 (3): 224–230)**

Key words: *sex; simvastatin; fenofibrate; atherogenic dyslipidaemia; monocytes; cytokines*

Streszczenie

Wstęp: Mimo stwierdzenia, że statyny i fibraty zmniejszają wydzielanie cytokin prozapalnych, nie ustalono jak dotąd, czy wpływ tych leków na funkcję sekrecyjną monocytów zależy od płci pacjenta.

Materiał i metody: W badaniu dokonano retrospektywnej analizy wyników 69 chorych na cukrzycę typu 2, dobranych pod względem wieku, masy ciała i stężenia lipidów, którzy z racji współistniejącej dyslipidemii aterogennej byli leczeni simwastatyną (40 mg dziennie), fenofibratem (200 mg dziennie) oraz terapią skojarzoną oboma lekami (w powyższych dawkach). Wydzielanie przez aktywowane monocyty czynnika martwicy nowotworów α (TNF-α), inteleukiny-1β, interleukiny 6 oraz białka chemotaktycznego dla monocytów-1 (MCP-1), jak również stężenie w osoczu białka C-reaktywnego (hsCRP) oraz wolnych kwasów tłuszczowych (FFA) oceniano oddzielnie dla kobiet i mężczyzn przed i po 12 tygodniach leczenia.

Wyniki: W warunkach wyjściowych wydzielanie cytokin prozapalnych oraz stężenie hsCRP i FFA były podobne u kobiet i mężczyzn. Simwastatyna, fenofibrat oraz łączne podawanie tych leków w zbliżonym stopniu hamowały uwalnianie przez aktywowane monocyty TNF-α, inteleukiny-1β, interleukiny-6 oraz MCP-1. Wpływ monoterapii simwastatyną i fenofibratem na wydzielanie cytokin prozapalnych oraz układowy stan zapalny nie różnił się pomiędzy kobietami i mężczyznami. W przypadku terapii skojarzonej obniżenie stężenia interleukiny-6 oraz MCP-1 było silniej wyrażone u mężczyzn. Zmiany sekrecji TNF-α oraz interleukiny-1β wykazywały również zależność od płci pacjenta, jednak różnica ta nie osiągała poziomu znamienności statystycznej.

Wnioski: Uzyskane wyniki badania sugerują, że płeć ma znaczenie dla wpływu łącznego podawania simwastatyny i fenofibratu na wydzielanie cytokin prozapalnych przez monocyty chorych z cukrzycą typu 2 i dyslipidemią aterogenną. **(Endokrynol Pol 2015; 66 (3): 224–230)**

Słowa kluczowe: *płeć; simwastatyna; fenofibrat; dyslipidemia aterogenna; monocyty; cytokiny*

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Abbreviations

ACCORD Lipid — the Action to Control Cardiovascular Risk in Diabetes Lipid
CRP — C-reactive protein
FFA — free fatty acids
FIELD — the Fenofibrate Intervention in Endpoint Lowering in Diabetes study
HDL — high-density lipoprotein
HMG-CoA — 3-hydroxy-3-methylglutaryl coenzyme A
HOMA-IR — the homeostatic model assessment of insulin resistance ratio
hsCRP — high-sensitivity C-reactive protein
LDL — low-density lipoprotein
PPAR-α — peroxisome proliferator-activated receptor-α
TNF-α — tumour necrosis factor α

Introduction

Although cardiovascular disease is the major cause of death in women over the age of 65 years, the risk of heart disease in women is often underestimated due to the misperception that females are 'protected' against this disease [1, 2]. The under-recognition of coronary artery disease and its often non-typical clinical picture in women result in less aggressive therapy and the underrepresentation of women in clinical trials [1]. Hypercholesterolaemia is a major factor contributing to the incidence of coronary artery disease. Recent guidelines recommend achieving the same low-density lipoprotein (LDL) cholesterol levels in men and women [3]. However, the clinical benefits associated with hypolipidaemic agents seem to result in part from their extralipid, so-called pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) and peroxisome proliferator-activated receptor-α (PPAR-α) activators (fibrates). These agents were found to produce anti-inflammatory, antioxidant, and antithrombotic properties, to regulate the growth and migration of smooth muscle cells and to improve endothelial function [4–7]. These effects may explain why statins are effective in reducing cardiovascular events in high-risk subjects without elevated LDL-cholesterol, including type 2 diabetes patients [8] and apparently healthy persons with elevated C-reactive protein (CRP) levels [9], whereas fibrates are effective in patients with low low-density lipoprotein (HDL) cholesterol and metabolic syndrome [10, 11].

In the Action to Control Cardiovascular Risk in Diabetes Lipid (ACCORD Lipid) trial, the addition of fenofibrate to statin therapy was associated with a 18% lower number of cardiovascular events in men, and a non-statistically significant 38% higher number of cardiovascular events in women [12]. However, although women exhibited slightly higher plasma lipids, higher frequency of non-white race, and less prior cardiovascular disease and microvascular disease, these differences do not seem to explain the significant differences in the primary outcome between men and women [12].

Nonetheless, subgroup analyses in the ACCORD Lipid [12] and the Fenofibrate Intervention in Endpoint Lowering in Diabetes study (FIELD) [13] trials indicate that fenofibrate is of the greatest benefit in decreasing cardiovascular events in patients with atherogenic dyslipidaemia. Previously, we observed that simvastatin and fenofibrate exhibited a similar effect on the secretory function of human monocytes and lymphocytes and on systemic inflammation in type 2 diabetic subjects with mixed dyslipidaemia [14]. In the present study, we decided to investigate whether statin and/or fibrate action on cytokine release by activated monocytes in patients with diabetes mellitus and atherogenic dyslipidaemia is sex-dependent.

Material and methods

We retrospectively analysed samples obtained from 85 subjects (20–75 years old) with type 2 diabetes and atherogenic dyslipidaemia, who had been complying with lifestyle intervention and had been treated with metformin at the daily dose of 1.7–3.0 g, but not with any hypolipidaemic patients, for at least 12 weeks before the beginning of the study. Type 2 diabetes was defined as fasting plasma glucose at least 126 mg/dL or plasma glucose concentration 2 hours after a glucose load of at least 200 mg/dL; while atherogenic dyslipidaemia was defined as HDL cholesterol levels below 40 mg/dL in men and 50 mg/dL in women, and triglycerides at least 150 mg/dL. Some of these patients were included in our previous study [14] and were eligible for the study, if they met the criteria of atherogenic dyslipidaemia. We excluded subjects with other forms of dyslipidaemias, any acute and chronic inflammatory processes, autoimmune disorders, unstable coronary artery disease, myocardial infarction or stroke within 6 months preceding the study, untreated stage 2 or 3 hypertension (according to the 2003 European Society of Hypertension–European Society of Cardiology guidelines), symptomatic congestive heart failure, body mass index above 35 kg/m^2, impaired renal or hepatic function, or malabsorption syndromes, as well as patients treated with insulin, oral antidiabetic drugs (with the exception of metformin), drugs known either to affect plasma lipid levels or to interact with statins and fibrates and with drugs that may affect inflammatory processes in the vascular wall (including non-steroid anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, and

angiotensin II receptor blockers) within the 3 months preceding the study.

The study protocol was approved by our institutional review board, and subjects gave written, informed consent to participate in the study. Simvastatin (40 mg daily), micronised fenofibrate (200 mg daily), or simvastatin (40 mg daily) plus fenofibrate (200 mg daily) were administered once daily at bedtime for 12 weeks, and no changes in medication dosage were allowed throughout the study. To minimise the risk of eventual pharmacokinetic interactions between simvastatin and fenofibrate, both drugs were administered in 12-hour time intervals (between 8.00 and 9.00 a.m., and between 8.00 and 9.00 p.m.). Compliance was monitored twice monthly by the number of tablets returned. During the study, all participants were also asked to follow lifestyle modification, the goals of which were a reduction in weight of at least 7%, total fat intake less than 30% of total energy intake, saturated fat intake less than 7% of energy consumed, cholesterol intake less than 200 mg per day, an increase in fibre intake to 15 g per 1000 kcal, and moderate to vigorous exercise for at least 30 minutes per day.

Laboratory investigations were performed at baseline and at the end of the treatment. Venous blood samples were taken after a 12-hour overnight fast, in a quiet, temperature-controlled room (24–25°C) between 8.00 and 9.00 a.m. (to avoid possible circadian fluctuations in the parameters studied). Analysis was performed by a person blinded to subject identity and all clinical details. Total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, glucose, and insulin were assessed by routine laboratory techniques (bioMerieux France; Incstar Corporation, Stillwater, MN, USA; Beckman, Palo Alto, CA, USA; Linco Research Inc., St Charles, MO, USA). LDL levels were measured directly. Fasting plasma glucose and insulin levels were used to calculate the homeostatic model assessment — insulin resistance index (HOMA-IR) [fasting serum glucose (mg/dL) × fasting insulin level (mU/mL)/405]. Total non-esterified free fatty acids (FFA) were assessed by an enzymatic assay using reagents from Alpha Laboratories (Eastleigh, Hants, UK). Circulating levels of CRP were determined using a high-sensitivity monoclonal antibody assay (hsCRP) (MP Biomedicals, Orangeburg, NY). Glycated haemoglobin was determined using a DCA 2000 analyser (Bayer Ames Technicon, Tarrytown, NY, USA). Cultures of phytohemagglutinin-stimulated T cells and lipopolysaccharide-stimulated monocytes were performed in triplicate as described previously [15, 16]. Monocyte release of TNF-α, inteleukin-1β, interleukin-6, and MCP-1 were estimated using commercial ELISA kits (R&D Systems, McKinley Place N.E. Minneapolis, MN), according to the manufacturer's instructions. The minimum detectable levels for the assessed cytokines were: 4.4 pg/mL (TNF-α), 1.0 pg/mL (inteleukin-1β), 3.8 pg/mL (interleukin-6), and 5.0 pg/mL (MCP-1). Intra- and inter-assay coefficients of variation were less than 5.8 and 8.6%, respectively.

The Kolmogorov-Smirnov test was used as the first statistical analysis approach to verify data distribution normality. Because of skewed distributions, values for the HOMA-IR, triglycerides, hsCRP, and cytokines were natural-log transformed to meet the assumptions of parametric tests. Comparisons between the groups were made by the *t* test for independent samples. Student's paired *t*-test was applied to compare pre- and post-treatment data within the same group. Correlations between the study parameters were calculated using Kendall's t test. Statistical significance was assumed at $p < 0.05$.

Results

In order to obtain age-, weight-, and plasma lipid-matched populations of men and women, in the present study, we chose to analyse the samples of 69 patients: 12 men and 11 women treated with simvastatin, 12 men and 10 women receiving fenofibrate, and 14 men and 10 women treated with simvastatin/ /fenofibrate combination. The data of the remaining patients participating in the study were not included in the final analyses. At study entry there were also no differences between the populations of men and women in terms of medical background and safety parameters (Table I). Both populations differed in waist circumference. Baseline monocyte release of TNF-α, interleukin-1β, interleukin-6, and MCP-1, as well as plasma hsCRP and FFA levels did not differ between the men and the women.

Neither drop-outs nor serious adverse events were observed throughout the study in the analysed groups of patients. All safety parameters remained within normal limits.

In both diabetic men and women with atherogenic dyslipidaemia complying with lifestyle modification and treated with metformin, simvastatin (Table II), fenofibrate (Table III), and simvastatin/fenofibrate combination therapy (Table IV) decreased total cholesterol, LDL cholesterol and triglycerides, plasma hsCRP and FFA, and monocyte release of TNF-α, interleukin-1β, interleukin-6 and MCP-1, as well as increased HDL cholesterol. Moreover, fenofibrate alone or in combination with simvastatin reduced plasma glucose, HOMA-IR, and glycated haemoglobin.

If the patients were treated exclusively with simvastatin and fenofibrate, the monocyte-suppressing effect was similar for men and women (Tables II and III). In

Table I. *Baseline characteristics of the participants*
Tabela I. *Wyjściowa charakterystyka pacjentów*

	Women	Men
Number of patients	31	38
Age [years; mean (SD)]	53.0 (2.3)	53.4 (2.4)
Smokers (%)	19	21
Body mass index [kg/m^2; mean (SD)]	28.1 (3.0)	28.7 (2.9)
Waist circumference [cm; mean (SD)]	95 (5)	101 (6)**
Grade 1 hypertension (%)	68	73
Intima-media thickness [mm; mean (SD)]	0.91 (0.21)	0.94 (0.18)
Total cholesterol [mg/dL; mean (SD)]	210 (19)	218 (16)
LDL cholesterol [mg/dL; mean (SD)]	121 (12)	128 (14)
HDL cholesterol [mg/dL; mean (SD)]	38 (4)	34 (4)
Triglycerides [mg/dL; mean (SD)]	228 (31)	240 (35)
Glucose [mg/dL; mean (SD)]	146 (11)	148 (12)
HOMA-IR [mean (SD)]	9.8 (1.1)	10.5 (1.3)
Glycated haemoglobin [%; mean (SD)]	7.0 (0.5)	7.2 (0.6)
hsCRP [mg/L; mean (SD)]	3.4 (0.6)	3.2 (0.5)
FFA [μmol/L; mean (SD)]	416 (57)	447 (61)
TNF-α [pg/mL; mean (SD)]	1842 (240)	1710 (285)
Interleukin-1β [pg/mL; mean (SD)]	143 (19)	155 (26)
Interleukin-6 [ng/mL; mean (SD)]	10.1 (3.0)	11.2 (2.8)
MCP-1 [ng/mL; mean (SD)]	20.4 (3.8)	22.2 (3.4)

**p < 0.01 vs. women

Table II. *The effect of 12-week simvastatin treatment on plasma lipids, glucose metabolism markers, and circulating levels of high sensitivity CRP, free fatty acids, and monocyte cytokine release in type 2 diabetic men and women with atherogenic dyslipidaemia*

Tabela II. *Wpływ 12-tygodniowego stosowania simwastatyny na stężenie lipidów, markery gospodarki węglowodanowej, stężenie CRP i wolnych kwasów tłuszczowych oraz wydzielanie cytokin prozapalnych przez aktywowane monocyty kobiet i mężczyzn z cukrzycą typu 2 oraz dyslipidemią aterogenną*

	Women (n = 11)	Men (n = 12)
ΔTotal cholesterol [mg/dL; mean (SD)]	–54 (18)***	–49 (15)***
ΔLDL cholesterol [mg/dL; mean (SD)]	–42 (14)***	–39 (12)***
ΔHDL cholesterol [mg/dL; mean (SD)]	7 (4)*	5 (3)*
ΔTriglycerides [mg/dL; mean (SD)]	–31 (18)*	–37 (20)*
ΔGlucose [mg/dL; mean (SD)]	–5 (7)	–5 (6)
ΔHOMA-IR [%; mean (SD)]	–1.0 (0.9)	–1.2 (1.1)
ΔGlycated haemoglobin [%; mean (SD)]	–0.2 (0.4)	–0.1 (0.4)
ΔhsCRP [mg/L; mean (SD)]	–1.6 (0.5)***	–1.3 (0.5)***
ΔFFA [μmol/L; mean (SD)]	–156 (43)***	–169 (39)***
ΔTNF-α [pg/mL; mean (SD)]	–639 (178)**	–680 (120)**
ΔInterleukin-1β [pg/mL; mean (SD)]	–58 (6)***	–64 (7)***
ΔInterleukin-6 [ng/mL; mean (SD)]	–3.4 (0.7)**	–3.5 (0.6)**
ΔMCP-1 [ng/mL; mean (SD)]	–4.8 (1.2)**	–5.6 (1.6)**

*p < 0.05; **p < 0.01; ***p < 0.001 post-treatment *vs.* baseline value

the case of simvastatin/fenofibrate combination therapy, the treatment-induced changes in monocyte release of interleukin-6 and MCP-1 were more pronounced in the men than in the women (Table IV). The impact of simvastatin/fenofibrate combination therapy on TNF-α, interleukin-1β and hsCRP was also stronger in the male population, but the difference did not reach the level of significance (p = 0.086 for TNF-α, p = 0.075 for interleukin-1β, and p = 0.096 for hsCRP).

In both men and women, simvastatin/fenofibrate combination therapy stronger than fenofibrate reduced circulating levels of total cholesterol, LDL cholesterol and triglycerides, as well as was superior to simvastatin in affecting total cholesterol, HDL cholesterol triglycerides, glucose and HOMA-IR, and glycated haemoglobin (Table IV). Irrespectively of sex, there were no differences between simvastatin and fenofibrate in the strength of their action on monocyte release of the investigated cytokines. Irrespectively of sex, the effect of combination therapy on plasma hsCRP was more pronounced than that of simvastatin or fenofibrate alone. In the men, the effect of simvastatin/fenofibrate combination therapy on interleukin-6 and MCP-1 was stronger than in the male patients treated with only one of these drugs (Table IV).

At entry, in both sexes, there was a correlation between cytokine release and plasma hsCRP levels (men: r values between 0.30 [p < 0.05] and 0.43 [p < 0.01]; women: r values between 0.35 [p < 0.05] and 0.47 [p < 0.001]) as well as between cytokine release and HOMA-IR (men: r values between 0.37 [p < 0.01] and 0.48 [p < 0.001]; women: r values between 0.39 [p < 0.05] and 0.47 [p < 0.01]).

In men and women the effect of hypolipidaemic treatment on monocyte release and plasma hsCRP did not correlate with the treatment-induced changes in plasma lipids. In both sexes there were correlations between the impact of hypolipidaemic treatment on cytokine release and its effect on hsCRP (simvastatin — men: r values between 0.46 [p < 0.01] and 0.59 [p < 0.001], women: r values between 0.43 [p < 0.01] and 0.58 [p < 0.001]; fenofibrate — men: r values between 0.43 [p < 0.01] and 0.57 [p < 0.001], women: r values between 0.43 [p < 0.01] and 0.55 [p < 0.001]; combination therapy: men: r values between 0.46 [p < 0.01] and 0.62 [p < 0.001], women: r values between 0.49 [p < 0.001] and 0.60 [p < 0.001]). The treatment-induced changes in monocyte release of TNF-α, interleukin-1β, interleukin-6 and MCP-1 release and plasma hsCRP

Table III. *The effect of 12-week fenofibrate treatment on plasma lipids, glucose metabolism markers, and circulating levels of high sensitivity CRP, free fatty acids, and monocyte cytokine release in type 2 diabetic men and women with atherogenic dyslipidaemia*

Tabela III. *Wpływ 12-tygodniowego stosowania fenofibratu na stężenie lipidów, markery gospodarki węglowodanowej, stężenie CRP i wolnych kwasów tłuszczowych oraz wydzielanie cytokin prozapalnych przez aktywowane monocyty kobiet i mężczyzn z cukrzycą typu 2 oraz dyslipidemią aterogenną*

	Women (n = 10)	Men (n = 12)
ΔTotal cholesterol [mg/dL; mean (SD)]	–27 (10)*	–25 (8)*
ΔLDL cholesterol [mg/dL; mean (SD)]	–20 (8)*	–21 (9)*
ΔHDL cholesterol [mg/dL; mean (SD)]	9 (5)**	9 (4)**
ΔTriglycerides [mg/dL; mean (SD)]	–65 (17)***	–60 (19)***
ΔGlucose [mg/dL; mean (SD)]	–14 (6)**	–15 (7)**
ΔHOMA-IR [%; mean (SD)]	–4.1 (0.9)**	–4.3 (1.1)***
ΔGlycated haemoglobin [%; mean (SD)]	–0.8 (0.6)**	–0.7 (0.5)**
ΔhsCRP [mg/L; mean (SD)]	–1.6 (0.5)***	–1.2 (0.4)***
ΔFFA [μmol/L; mean (SD)]	–192 (31)***	–176 (26)***
ΔTNF-α [pg/mL; mean (SD)]	–570 (172)**	–601 (196)**
ΔInterleukin-1β [pg/mL; mean (SD)]	–60 (12)***	–68 (11)***
ΔInterleukin-6 [ng/mL; mean (SD)]	–3.2 (0.5)**	–3.5 (0.6)**
ΔMCP-1 [ng/mL; mean (SD)]	–5.7 (1.0)**	–6.5 (1.3)**

*p < 0.05; **p < 0.01; ***p < 0.001 post-treatment *vs.* baseline value

Table IV. *The effect of 12-week simvastatin/fenofibrate combination therapy on plasma lipids, glucose metabolism markers, and circulating levels of high sensitivity CRP, free fatty acids, and monocyte cytokine release in type 2 diabetic men and women with atherogenic dyslipidaemia*

Tabela IV. *Wpływ 12-tygodniowego stosowania simwastatyny wraz z fenofibratem na stężenie lipidów, markery gospodarki węglowodanowej, stężenie CRP i wolnych kwasów tłuszczowych oraz wydzielanie cytokin prozapalnych przez aktywowane monocyty kobiet i mężczyzn z cukrzycą typu 2 oraz dyslipidemią aterogenną*

	Women (n = 10)	Men (n = 14)
ΔTotal cholesterol [mg/dL; mean (SD)]	–67 (14)***ᵍ^^^	–70 (12)***ᵍ^^^
ΔLDL cholesterol [mg/dL; mean (SD)]	–43 (12)***^^^	–47 (11)***^^^
ΔHDL cholesterol [mg/dL; mean (SD)]	13 (6)***ᵍ	11 (5)***ᵍ
ΔTriglycerides [mg/dL; mean (SD)]	–85 (15)***ᵍᵍᵍ^	–79 (13)***ᵍᵍᵍ^
ΔGlucose [mg/dL; mean (SD)]	–13 (4)**ᵍ	–14 (5)**ᵍ
ΔHOMA-IR [%; mean (SD)]	–3.8 (0.8)***ᵍᵍᵍ	–4.1 (1.0)***ᵍᵍᵍ
ΔGlycated haemoglobin [%; mean (SD)]	–0.8 (0.7)**ᵍ	–0.9 (0.6)**ᵍ
ΔhsCRP [mg/L; mean (SD)]	–2.3 (0.5)***ᵍᵍ^^	–1.9 (0.4)***ᵍᵍ^^
ΔFFA [μmol/L; mean (SD)]	–189 (41)***	–210 (37)***
ΔTNF-α [pg/mL; mean (SD)]	–620 (202)***	–780 (155)***
ΔInterleukin-1β [pg/mL; mean (SD)]	–58 (14)***	–68 (15)***
ΔInterleukin-6 [ng/mL; mean (SD)]	–3.6 (0.7)***	–4.6 (0.7)***#ᵍ^
ΔMCP-1 [ng/mL; mean (SD)]	–6.1 (1.9)***	–8.3 (2.1)***#ᵍ^

**p < 0.01; ***p < 0.001 post-treatment *vs.* baseline value
#p < 0.05 *vs.* women
ᵍp < 0.05; ᵍᵍp < 0.01; ᵍᵍᵍp < 0.001 *vs.* the effect of simvastatin in the patients of the same sex
^p < 0.05; ^^p < 0.01; ^^^p < 0.001 *vs.* the effect of fenofibrate in the patients of the same sex

correlated with the degree of reduction in FFA (simvastatin — men: r values between 0.32 [p < 0.05] and 0.55 [p < 0.001], women: r values between 0.34 [p < 0.05] and 0.52 [p < 0.001]; fenofibrate — men: r values between 0.44 [p < 0.01] and 0.58 [p < 0.001], women: r values between 0.46 [p < 0.01] and 0.56 [p < 0.001]; combination therapy: men: r values between 0.47 [p < 0.01] and 0.61 [p < 0.001], women: r values between 0.49 [p < 0.001] and 0.59 [p < 0.001]), and in the case of fenofibrate and simvastatin/fenofibrate combination therapy with the effect on HOMA-IR (fenofibrate — men: r values between 0.35 [p < 0.05] and 0.50 [p < 0.001], women: r values between 0.31 [p < 0.05] and 0.51 [p < 0.001]; combination therapy — men: r values between 0.37 [p < 0.01] and 0.60 [p < 0.001], women: r values between 0.40 [p < 0.01] and 0.56 [p < 0.001]).

Discussion

Probably the most important finding of our study is that the effect of simvastatin/fenofibrate combination therapy on monocyte release of proinflammatory cytokines is more pronounced in men than in women with type 2 diabetes and atherogenic dyslipidaemia. In the whole population of investigated patients, the monocyte-suppressing effect was similar in the patients treated with statin/fenofibrate combination therapy and in those receiving only one of these drugs. But only in the men, the effect of simvastatin/fenofibrate combination therapy on interleukin-6 and MCP-1 was more pronounced than that of simvastatin or ezetimibe administered alone.

These findings may partially explain why, in the ACCORD Lipid trial, fenofibrate added to a HMG-CoA reductase inhibitor reduced cardiovascular risk exclusively in men [12]. Interestingly, in the remaining treatment groups, sex differences did not determine the effect of hypolipidaemic therapy on monocyte secretory

function and low-grade inflammation, and in line with this observation the impact of statins [8, 9] and fibrates [10, 11, 13] on the investigated end-points in other large clinical trials did not differ between men and women.

All participants of our study met the criteria of atherogenic dyslipidemia and these individuals may be the best candidates for statin/fibrate combination therapy. In the ACCORD Lipid trial, patients with HDL cholesterol below 34 mg/dL and triglycerides above 204 mg/dL gained the greatest clinical benefit from adding fenofibrate to statin therapy [12]. Certainly, there are some important differences between our study and the ACCORD Lipid trial. Our study included exclusively subjects with recently diagnosed and previously untreated dyslipidaemia, who were at once simultaneously treated with simvastatin and fenofibrate. In turn, in the mentioned clinical trial, diabetes lasted on average 10 years, dyslipidaemia had not always been present before the beginning of the study, participants suffered from various coexisting disorders, had already been treated with a HMG-CoA reductase inhibitor, and statin doses differed between patients.

Baseline monocyte cytokine release did not differ between the men and women, and this finding is in contrast to the findings of other authors [17–20] who observed higher monocyte production and plasma levels of proinflammatory cytokines in men. These differences may be explained by various inclusion criteria, which were very strict in our study. The male and female participants of our study were characterised by similar clinical and laboratory characteristics (with the exception of waist circumference), which enabled us to reduce the possible impact of other disorders or concomitant therapies. Alternatively, as lymphocyte release of proinflammatory cytokines is sex-dependent [Krysiak et al., unpublished observations], higher circulating levels of proinflammatory cytokines in men may reflect sex-dependent differences in the function of other types of cells.

A complex proatherogenic action of TNF-α, inteleukin-1β, interleukin-6, and MCP-1 [21, 22], as well as the fact that monocytes are the primary inflammatory cell type that infiltrate early atherosclerotic plaques, playing a variety of roles in atherosclerotic plaque development and its clinical sequelae [23, 24], suggest that the monocyte-suppressing effect of simvastatin and fenofibrate administered alone or in combination may be clinically relevant for both men and women. Considering that even small differences in the investigated variables may be related to various risks of cardiovascular disorders, as it was found in the case of TNF-α and interleukin-1β, combined administration of a statin and a fibrate may bring important clinical benefits to type 2 diabetic men with atherogenic dyslipidaemia, who have high cardiovascular risk [25].

The lack of a correlation between the effect on cytokine release and the extent of lipid-lowering action of simvastatin and/or fenofibrate, shown in both sexes, indicates that the impact on monocyte secretory function belongs to the pleiotropic effects of these agents. This effect, irrespectively of gender, is reciprocally interrelated with their action on systemic inflammation, and the latter action is probably partially secondary to statin- and/or fibrate-induced changes in monocyte secretory function.

Irrespectively of gender, the effect of hypolipidaemic agents on monocyte cytokine release and systemic inflammation correlated with their action on FFA and, in the patients treated with fenofibrate or simvastatin/fenofibrate combination therapy, with an improvement in insulin sensitivity. In the case of fenofibrate, the presence of these correlations is a logical consequence of the involvement of FFA in the development of insulin resistance in both men and women [26], as well as the role of endogenous ligands for PPAR-α [27]. Finding that a similar reduction in FFA was induced by simvastatin is in agreement with the hypothesis that the molecular mechanisms of the action of statins and fibrates partially overlap [28, 29]. An inhibitory effect of simvastatin-induced inhibition of protein prenylation on the activity of glucose transporter 4 [30], responsible for insulin-regulated glucose transport into the cell [31], may explain why, despite a reduction in FFA, simvastatin did not improve insulin sensitivity.

Interestingly, the effect of simvastatin and fenofibrate on cytokine release correlated better with the clinical outcome of the ACCORD Lipid trial than their action on circulating levels of hsCRP. Although this protein is regarded as one of the most significant risk factors for cardiovascular disease and is directly involved in atherogenesis [32], unlike the results of the ACCORD Lipid trial, the effect of simvastatin and fenofibrate on hsCRP was additive in both sexes, and only insignificantly stronger in the men than the women.

This study has some limitations. Firstly, the study included only a limited number of patients and measured only surrogates of outcome, and hence the findings must be interpreted with caution. Secondly, our study was a retrospective analysis of the stored samples. Furthermore, all participants were treated with metformin, and therefore it is not certain whether the investigated treatment options produce similar effects in patients treated with other hypoglycaemic agents. Finally, all our patients had coexistent type 2 diabetes. Therefore, the question of whether a similar effect is observed in insulin-resistant patients with less expressed glucose metabolism abnormalities remains open.

Conclusions

Our study has shown for the first time that the effect of simvastatin/fenofibrate combination therapy, but not of simvastatin or fenofibrate administered alone, on monocyte cytokine release in type 2 diabetes patients with atherogenic dyslipidaemia differs between men and women. This finding, requiring confirmation in future studies, suggests that sex differences partially determine the strength of the monocyte-suppressing effect of simvastatin/fenofibrate combination therapy in this group of patients.

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