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The effect of hypolipidaemic treatment on monocyte release of proinflammatory cytokines in different age groups of patients with type 2 diabetes and atherogenic dyslipidaemia

Wpływ leczenia hipolipemicznego na wydzielanie cytokin prozapalnych w różnych grupach wiekowych pacjentów z cukrzycą typu 2 i dyslipidemią aterogenną

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Abstract

Introduction: Although both statins and fibrates have been found to reduce monocyte cytokine release, no study has investigated whether the effect of hypolipidaemic agents depends on age.

Materials and methods: This study retrospectively analysed the results of 65 patients with type 2 diabetes and atherogenic dyslipidaemia, complying with lifestyle intervention, and receiving metformin. These patients were then treated with simvastatin (40 mg daily), micronized fenofibrate (200 mg daily), or simvastatin plus fenofibrate. Tumour necrosis factor-α (TNF-α), inteleukin-1β, interleukin-6, and monocyte chemoattractant protein-1 (MCP-1) release, as well as circulating levels of high-sensitivity C-reactive protein (hsCRP), were determined separately for patients aged between 20 and 50 years and between 51 and 75 years before the study and after 12 weeks of hypolipidaemic treatment.

Results: Older adults were characterised by higher monocyte release of TNF-α and interleukin-6, as well as higher circulating levels of hsCRP, than the younger subjects. The decrease in monocyte release of all investigated cytokines and in plasma hsCRP was similar in both age groups. In turn, the effect of fenofibrate, alone or in combination with simvastatin, on TNF-α, interleukin-6, and hsCRP, but not on interleukin-1β and MCP-1, was stronger in patients aged between 50 and 75 years, and correlated with an improvement in insulin sensitivity only in this age group.

Conclusions: Our results suggest that age may partially determine monocyte-suppressing and systemic anti-inflammatory effects of fenofibrate. **(Endokrynol Pol 2016; 67 (2): 190–196)**

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

Streszczenie

Wstęp: Chociaż zarówno statyny, jak i fibraty wydają się zmniejszać wydzielanie cytokin prozapalnych, jak dotąd nie badano, czy wpływ tych leków na funkcję sekrecyjną monocytów zmienia się z wiekiem pacjenta.

Materiał i metody: Badanie stanowiło retrospektywną analizę wyników 65 chorych na cukrzycę typu 2 i dyslipidemię aterogenną, przestrzegających zaleceń zmiany stylu życia i leczonych metforminą. Pacjenci ci byli następnie leczeni simwastatyną (40 mg dziennie), fenofibratem (200 mg dziennie) lub terapią skojarzoną oboma lekami (w powyższych dawkach). Ocenę wydzielania czynnika martwicy nowotworów α (TNF-α), inteleukiny-1β, interleukiny 6 oraz białka chemotaktycznego dla monocytów-1 (MCP-1) przez aktywowane monocyty, jak również ocenę stężenia w osoczu białka C-reaktywnego (hsCRP) porównywano pomiędzy chorymi w wieku 20–50 lat oraz 51–75 lat w warunkach wyjściowych i po 12 tygodniach stosowania terapii hipolipemicznej.

Wyniki: Starsza grupa wiekowa charakteryzowała się wyższym wydzielaniem TNF-α i interleukiny-6, jak również wyższym stężeniem hsCRP. U chorych otrzymujących simwastatynę hamujący wpływ tego leku na sekrecję cytokin prozapalnych i stężenie hsCRP był podobny w obu przedziałach wiekowych. Z kolei wpływ fenofibratu oraz terapii skojarzonej na wydzielanie TNF-α i interleukiny-6 oraz stężenie hsCRP, ale nie na sekrecję inteleukiny-1β i MCP-1 był bardziej wyrażony w starszej grupie wiekowej i jedynie w tej grupie korelował z poprawą wrażliwości na insulinę.

Wnioski: Wyniki badania sugerują, że wiek pacjenta może odgrywać rolę dla siły działania fenofibratu na funkcję sekrecyjną monocytów u chorych z cukrzycą typu 2 i dyslipidemią aterogenną. **(Endokrynol Pol 2016; 67 (2): 190–196)**

Słowa kluczowe: *wiek; 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
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

The prevalence of diabetes and lipid metabolism abnormalities increases with age, and cardiovascular disease, being a frequent complication of metabolic disorders, is a leading cause of death of older adults in developed countries [1, 2]. One of the possible mechanisms explaining the relationship between age and these disorders is a proinflammatory state. Aging is characterised by a two- to four-fold increase in circulating levels of inflammatory mediators, particularly TNF-α, interleukin-6, and interleukin-1 [3]. All these cytokines exert a multi-directional proatherogenic effect, and their increased production may contribute to the development and progression of atherosclerosis [4, 5]. Aging is also associated with higher plasma levels of C-reactive protein (CRP), regarded as a sensitive marker of systemic inflammation of high prognostic value in the prediction of cardiovascular risk [6, 7].

Although large clinical trials have shown that 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors reduced cardiovascular morbidity and mortality [8, 9] the percentage of older adults included in these studies was small [10–12]. There is, however, some evidence, based on subgroup analyses, that statin therapy in elderly patients is as effective as in younger patients [10, 12]. Despite these obvious benefits, statin therapy seems to be associated with a slightly increased risk of development of diabetes [13, 14]. An improvement in glucose homeostasis was, in turn, induced by peroxisome proliferator-activated receptor-α (PPARα) activators (fibrates) [15–18], but the favourable effect of this group of hypolipidaemic agents on cardiovascular morbidity and mortality was observed only in some studies [19, 20]. Moreover, no large clinical study has investigated the relationship between age and clinical effectiveness of PPARα activators or included only older adults.

As well as lowering plasma lipids, both HMG-CoA reductase inhibitors and PPAR-α activators exert anti-inflammatory, antioxidant, and antithrombotic properties, and improve endothelial function [21–24] and both lipid- and non-lipid-related mechanisms participating in the reduction of cardiovascular endpoints in statin [8, 9] and some fibrate trials [19, 20]. Interestingly, in one of our previous studies, simvastatin and fenofibrate reduced monocyte cytokine release but this effect was not stronger if both drugs were administered together [18]. This effect on the secretory function of cells abundantly present in atherosclerotic lesions and playing a role in atherogenesis [25, 26] is in line with the results of the Action to Control Cardiovascular Risk in Diabetes Lipid trial (ACCORD) [27]. This large clinical trial revealed that a simvastatin-fenofibrate combination was equipotent to simvastatin alone in decreasing cardiovascular and cerebrovascular risk in middle-aged and elderly subjects with type 2 diabetes and coronary artery disease or increased cardiovascular risk. Moreover, in our recent study, the effect of simvastatin/fenofibrate combination therapy on monocyte release of interleukin-6 and MCP-1 was more pronounced in the male population [28]. This finding is also in agreement with the results of ACCORD [27], in which statin/fibrate combination therapy was superior to statin alone in men but not in women.

To the best of our knowledge, no previous study has investigated whether the effect of hypolipidaemic agents on monocyte secretory function and systemic inflammation is determined by age. The aim of our study was to compare the effects of simvastatin, fenofibrate, and simvastatin/fenofibrate combination therapy on monocyte cytokine release and low-grade inflammation between two different aged populations of patients with type 2 diabetes mellitus and dyslipidaemia.

Material and methods

Our study was a retrospective analysis of data of 65 patients (20–75 years old) with type 2 diabetes (fasting plasma glucose at least 126 mg/dL or plasma glucose concentration two hours after a glucose load of at least 200 mg/dL) and atherogenic dyslipidaemia. To be admitted to the study they (a) had be treated with metformin (1.7–2.55 g daily), (b) had to comply with the lifestyle modification (total fat intake < 30% of total energy intake, saturated fat intake < 7% of energy consumed, cholesterol intake < 200 mg per day, an increase in fibre intake to 15 g per 1,000 kcal, moderate to vigorous exercise for at least 30 minutes per day) for at least 12 weeks before the beginning of the study; and (c) were required to have HDL cholesterol levels below 40 mg/dL in men and 50 mg/dL in women, and triglycerides at least 150 mg/dL. Of 65 analysed patients, 46 individuals participated in the previous study [18].

However, because they met all inclusion criteria and the study protocol that they followed was exactly the same as that required in the present study, their results were included in our analysis. In turn, the results of 53 patients were used by our research group in an analysis investigating whether sex differences determine the strength of monocyte-suppressing and systemic anti-inflammatory effects of simvastatin, fenofibrate, and simvastatin/fenofibrate combination therapy in type 2 diabetic patients with atherogenic dyslipidaemia [28].

The study excluded patients with type 1 diabetes, other forms of dyslipidaemias, autoimmune disorders, any acute and chronic inflammatory processes, unstable coronary artery disease, myocardial infarction or stroke within six 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, and malabsorption syndromes. In most patients, type 1 diabetes was excluded based on personal history and clinical picture. In case of any doubts we measured circulating levels of C-peptide, and titres of glutamic acid decarboxylase, islet cell, and insulinoma-associated-2 autoantibodies. No patient was allowed to receive antidiabetic (with the exception of metformin) or hypolipidaemic agents, drugs interacting with statins or fibrates, as well as drugs known to affect inflammatory processes in the vascular wall (including non-steroid anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers) within three months before the study onset.

The study protocol was approved by the local ethics committee. Informed consent was obtained from all enrolled participants. During the study, all participants, as well as complying with the lifestyle modification and metformin treatment (at the same dose as before the study) were treated with constant doses of simvastatin (40 mg daily), micronised fenofibrate (200 mg daily), or simvastatin (40 mg daily) plus fenofibrate (200 mg daily). Both drugs were administered once daily for 12 weeks. Based on age, each treatment group was divided into two subgroups: patients aged between 20 and 50 years and patients aged between 51 and 75 years. Compliance was monitored during each visit by tablet count.

Venous blood samples were collected from patients twice, at the beginning and at the end of the treatment period. Blood was drawn in the morning between 8.00 and 9.00 a.m. to avoid circadian variations in the parameters studied after an overnight fast. All assays were made in duplicate to minimise analytical errors. Total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides were measured colourimetrically using commercially available kits obtained from bioMerieux (France). Circulating glucose levels were analysed by a glucose oxidase method (Beckman, Palo Alto, CA, USA), and plasma insulin concentration was measured with a commercial radioimmunoassay kit (Linco Research Inc., St Charles, MO, USA). To avoid mistakes resulting from the Friedewald formula, LDL cholesterol was determined directly. Insulin resistance was assessed through the homeostatic model assessment — insulin resistance index (HOMA-IR) using the following equation: [fasting glucose (mg/dL) × fasting insulin (μU/mL)/405]. Glycated haemoglobin was measured by DCA 2000 (Bayer Ames Technicon, Tarrytown, NY, USA) standardised to Diabetes Control and Complications Trial standards. Plasma levels of CRP were determined using a high-sensitivity monoclonal antibody assay (hsCRP) (MP Biomedicals, Orangeburg, NY, USA). Lipopolysaccharide-stimulated monocytes were cultured in triplicate, as previously described [29, 30]. Monocyte release of TNF-α, inteleukin-1β, interleukin-6, and MCP-1 was measured using commercial enzyme-linked immunosorbent assay kits (R&D Systems, McKinley Place N.E. Minneapolis, MN), according to the manufacturer's instructions. The minimum detectable levels for the assessed variables were: 0.1 ng/mL (hsCRP), 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 interassay coefficients of variation were less than 5.8 and 8.6%, respectively.

The Kolmogorov-Smirnov test was used to evaluate data for underlying assumptions of normality. Values for HOMA-IR, triglycerides, hsCRP, and cytokines were natural log transformed to yield a normal distribution for statistical analyses. Student's *t*-test for independent samples was used to determine whether any statistically significant difference existed between both age populations, while Student's paired *t* test was used to investigate the significance of change within the same group. The chi^2 test was used for categorical data, and Kendall's tau test was used to determine the significance of each correlation. A value of $p < 0.05$ was considered statistically significant.

Results

To obtain sex-, weight-, and plasma lipid-matched populations of younger and older adults, we analysed the samples obtained from 65 patients, treated with simvastatin (n = 22), fenofibrate (n = 21) or simvastatin/ /fenofibrate combination therapy (n = 22), and only data of these patients were included in the final analyses. At baseline, both groups were comparable with respect to sex, smoking habits, blood pressure, body mass index, plasma lipids, plasma glucose, and glycated haemoglobin (Table I). The older adults were characterised

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

	Younger adults[1] (n = 30)	Older adults[2] (n = 35)
Number of patients	30	35
Age (years; mean [SD])	41 (4)	61 (5)[b]
Smokers (%)	20	20
Body mass index [kg/m^2; mean (SD)]	27.8 (2.5)	28.8 (3.0)
Grade 1 hypertension (%)	73	69
Total cholesterol [mg/dL; mean (SD)]	215 (20)	220 (21)
LDL cholesterol [mg/dL; mean (SD)]	131 (14)	135 (17)
HDL cholesterol [mg/dL; mean (SD)]	35 (4)	34 (4)
Triglycerides [mg/dL; mean (SD)]	230 (39)	245 (40)
Glucose [mg/dL; mean (SD)]	144 (12)	149 (13)
HOMA-IR [mean (SD)]	9.6 (1.1)	10.7 (1.4)
Glycated haemoglobin (%; mean [SD])	7.1 (0.6)	7.4 (0.5)
hsCRP [mg/L; mean (SD)]	3.1 (0.7)	3.8 (0.7)[a]
TNF-α [pg/mL; mean (SD)]	1620 (265)	1925 (292)[a]
Interleukin-1β [pg/mL; mean (SD)]	139 (28)	153 (30)
Interleukin-6 [ng/mL; mean (SD)]	9.1 (2.6)	12.1 (2.9)[a]
MCP-1 [ng/mL; mean (SD)]	19.8 (4.1)	22.5 (3.9)

[1]20–50 years old; [2]51–75 years old; [a]$p < 0.05$, [b]$p < 0.001$ *vs.* younger adults

by higher circulating levels of hsCRP. Compared to monocytes of younger patients, lipopolysaccharide-stimulated monocytes of patients aged 50–75 years released larger amounts of TNF-α and inteleukin-6, but not of interleukin-1β and MCP-1.

Simvastatin, fenofibrate, and simvastatin/fenofibrate combination therapy were well tolerated and all patients completed the study protocol. All safety parameters (aminotransferases, creatine kinase, creatinine, and blood morphology) remained within normal limits.

Expectedly, simvastatin, fenofibrate, and simvastatin/fenofibrate combination therapy administered to patients with type 2 diabetes and atherogenic dyslipidaemia complying with lifestyle intervention and treated with metformin decreased total cholesterol, LDL cholesterol, and triglycerides and increased HDL cholesterol (Table II). Moreover, fenofibrate and simvastatin/fenofibrate combination therapy reduced HOMA-IR and fasting glucose. The effect of fenofibrate alone or in combination with simvastatin on HOMA-IR was stronger in the older than in the younger adults. In both age groups the effect of fenofibrate on HOMA-IR, glucose, glycated haemoglobin, and triglycerides was stronger, while the effect on total and LDL cholesterol was weaker than that of simvastatin (Table II).

Table II. *The effect of 12-week treatment with simvastatin, fenofibrate, and simvastatin/fenofibrate combination therapy on plasma lipids, glucose metabolism markers, low-grade inflammation, and monocyte release of the investigated cytokines in different age groups of patients with type 2 diabetes and atherogenic dyslipidaemia*

Tabela II. *Wpływ dwunastotygodniowego stosowania simwastatyny, fenofibratu oraz terapii skojarzonej na stężenie lipidów, markery gospodarki węglowodanowej, układowy stan zapalny oraz wydzielanie przez monocyty cytokin prozapalnych u chorych na cukrzycę typu 2 oraz dyslipidemię aterogenną*

	Simvastatin		Fenofibrate		Combination therapy	
	Younger adults[1] (n = 10)	Older adults[2] (n = 12)	Younger adults[1] (n = 10)	Older adults[2] (n = 11)	Younger adults[1] (n = 10)	Older adults[2] (n = 12)
ΔTotal cholesterol [mg/dL; mean (SD)]	–57 (20)[c, i]	–51 (18)[c, i]	–24 (8)[a]	–26 (9)[a]	–63 (16)[c, i]	–70 (12)[c, i]
ΔLDL cholesterol [mg/dL; mean (SD)]	–44 (16)[c, h]	–41 (13)[c, h]	–22 (7)[a]	–20 (8)[a]	–46 (14)[c, i]	–48 (15)[c, i]
ΔHDL cholesterol [mg/dL; mean (SD)]	8 (4)[a]	6 (4)[a]	10 (5)[b]	8 (4)[b]	12 (5)[c, d]	13 (5)[c, d]
ΔTriglycerides (mg/dL; mean [SD])	–39 (21)[a]	–35 (20)[a]	–61 (20)[b, d]	–56 (16)[b, d]	–82 (18)[c, f, g]	–80 (15)[c, f, g]
ΔGlucose [mg/dL; mean (SD)]	–5 (6)	–4 (5)	–12 (6)[a, d]	–14 (7)[a, d]	–12 (7)[a, d]	–13 (6)[a, d]
ΔHOMA-IR [mean (SD)]	0.1 (0.8)	0.3 (0.8)	–2.3 (0.8)[b, e]	–3.5 (1.1)[b, e, j]	–2.5 (0.9)[a, f]	–4.1 (1.0)[c, f, j]
ΔGlycated haemoglobin (%; mean [SD])	0.1 (0.3)	0.2 (0.2)	–0.5 (0.2)[a, d]	–0.7 (0.3)[a, d]	–0.8 (0.3)[b, d]	–0.6 (0.4)[b, d]
ΔhsCRP [mg/L; mean (SD)]	–1.4 (0.6)[b]	–1.4 (0.7)[b]	–1.1 (0.4)[a]	–1.7 (0.5)[a]	–1.7 (0.5)[b, g]	–2.3 (0.6)[c, d, g, j]
ΔTNF-α [pg/mL; mean (SD)]	–613 (180)[b]	–665 (245)[b]	–440 (254)	–751 (208)[b, j]	–702 (202)[c, g]	–960 (189)[c, d, g, j]
ΔInterleukin-1β [pg/mL; mean (SD)]	–59 (18)[b]	–57 (16)[b]	–56 (12)[b]	–64 (16)[b]	–55 (14)[c]	–65 (18)[c]
ΔInterleukin-6 [ng/mL; mean (SD)]	–3.0 (1.2)[a]	–3.5 (1.4)[a]	–2.5 (1.0)	–3.9 (1.2)[b, j]	–2.9 (0.9)[a]	–5.0 (1.5)[c, d, g, j]
ΔMCP-1 [ng/mL; mean (SD)]	–6.0 (1.1)[b]	–6.3 (1.4)[b]	–6.0 (1.5)[b]	–6.2 (1.8)[b]	–6.5 (1.7)[b]	–8.0 (2.3)[c]

[1]20–50 years old; [2]51–75 years old.; [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$ post-treatment *vs.* baseline value; [d]$p < 0.05$; [e]$p < 0.01$; [f]$p < 0.001$ *vs.* the effect of simvastatin in the same age group; [g]$p < 0.05$; [h]$p < 0.01$; [i]$p < 0.001$ *vs.* the effect of fenofibrate in the same age group; [j]$p < 0.05$ *vs.* the effect of the same treatment option in younger patients

Table III. *Correlations between the assessed variables*
Tabela III. *Korelacje pomiędzy ocenianymi markerami*

		Younger adults[1]	Older adults[2]
hsCRP	TNF-α	0.55* (0.39*, 0.48*, 0.55*)	0.57* (0.48*, 0.46*, 0.59*)
hsCRP	Interleukin-1β	0.38* (0.37*, 0.34*, 0.30*)	0.50* (0.39*, 0.42*, 0.51*)
hsCRP	Interleukin-6	0.47* (0.52*, 0.53*, 0.56*)	0.42* (0.51*, 0.58*, 0.47*)
hsCRP	MCP-1	0.35* (0.46*, 0.41*,0.47*)	0.37* (0.40*, 0.36*, 0.30*)
HOMA-IR	hsCRP	0.43* (0.05, 0.18, 0.23)	0.50* (0.12, 0.46*, 0.53*)
HOMA-IR	TNF-α	0.46* (0.11, 0.24, 0.21)	0.49* (0.16, 0.38*, 0.61*)
HOMA-IR	Interleukin-1β	0.38* (0.08, 0.22, 0.19)	0.42* (0.02, 0.22, 0.18)
HOMA-IR	Interleukin-6	0.40* (0.15, 0.20, 0.18)	0.43* (0.11, 0.59*, 0.34*)
HOMA-IR	MCP-1	0.29* (0.13, 0.23,0,21)	0.31* (0.12, 0.17, 0.19)
		All studied patients[3]	
Age	hsCRP	0.37* (0.11, 0.37*, 0.29*)	
Age	TNF-α	0.39* (0.14, 0.32*, 0.38*)	
Age	Interleukin-1β	0.21 (0.13, 0.19, 0.18)	
Age	Interleukin-6	0.34 (0.17, 0.38*, 0.37*)	
Age	MCP-1	0.20 (0.16, 0.20, 0.20)	

Data represent the correlation coefficients (r values) at baseline and in parentheses after treatment with simvastatin, fenofibrate, and combination therapy.
120–50 years old; 251–75 years old; 320–75 years old; *statistically significant

Simvastatin reduced monocyte release of TNF-α, inteleukin-1β, interleukin-6, and MCP-1, and decreased plasma hsCRP levels, and the strength of this action was similar in both age groups (Table II). Fenofibrate administered alone reduced monocyte release of interleukin-1β and MCP-1 in both younger and older patients. Although fenofibrate decreased TNF-α and inteleukin-6, this effect was statistically significant only in patients aged between 51 and 75 years and its strength differed between both age populations. It also reduced plasma hsCRP in both age groups, but this effect was more pronounced in the older age group (Table II). As this table also shows, the strongest effect on plasma lipids, hsCRP, and TNF-α and inteleukin-6 release was observed in older adults receiving both simvastatin and fenofibrate. Simvastatin/fenofibrate combination therapy decreased monocyte release of all investigated proinflammatory cytokines and circulating hsCRP levels in both age populations. However, its effect on TNF-α, inteleukin-1β, and hsCRP was more pronounced in patients aged between 51 and 75 years than between 20 and 50 years (Table II).

Correlations between the assessed variables are shown in Table III. At the beginning of the study, monocyte cytokine release correlated with plasma hsCRP levels, and as there were correlations between hsCRP or cytokine release and HOMA-IR. Moreover, baseline levels of hsCRP, TNF-α, and interleukin-6 correlated with age. Treatment-induced changes in cytokine release correlated with the impact of hypolipidaemic treatment on hsCRP The effect of fenofibrate and simvastatin/ /fenofibrate combination therapy on hsCRP, TNF-α, and interleukin-6 correlated with age. Moreover, in older adults there were correlations between the effects of fenofibrate and simvastatin/fenofibrate combination therapy on TNF-α and interleukin-6 release and hsCRP and on HOMA-IR. No other correlations were observed.

Discussion

This study shows for the first time that age may partially determine the effect of fenofibrate on cytokine release and low-grade inflammation. In line with this hypothesis, both fenofibrate alone and in combination with simvastatin reduced circulating levels of all cytokines measured in our study in patients aged between 51 and 75 years, while in the younger subjects its effect was limited to interleukin-1β and MCP-1. Moreover, the impact of fenofibrate on hsCRP was more pronounced in the former group of patients. In patients receiving combined hypolipidaemic therapy this effect cannot be explained by simultaneous use of a HMG-CoA reductase inhibitor because simvastatin alone reduced cytokine release and decreased hsCRP to a similar extent in both age populations. Taking into account the complex proatherogenic effect of TNF-α and interleukin-6 [4, 5], as well as the important role of monocytes/macrophages [25, 26] and low-grade in-

flammation [6, 7] in atherogenesis, the obtained results may be relevant. They suggest that older adults may receive greater benefit from PPAR-α activator therapy than younger ones, while the potency of HMG-CoA reductase inhibitors does not depend on the age.

Baseline levels of hsCRP, as well as monocyte release of TNF-α and inteleukin-6, were higher in older than in younger adults. These differences cannot be associated with various clinical characteristics of the participants because sex distribution, smoking habit, and blood pressure were similar in younger and older adults. They cannot be also attributed to increased body weight and more pronounced dyslipidaemia, as a consequence of aging [31, 32], because potential participants were initially preselected in order to obtain weight- and plasma lipid-matched groups. Moreover, higher levels of TNF-α, interleukin-6, and hsCRP persisted even after adjusting for body mass index and lipids (data not shown). However, the obtained results are probably, at least in part, associated with impaired insulin receptor action because monocyte release of TNF-α, interleukin-6, as well as plasma hsCRP levels correlated with HOMA-IR. Interestingly, TNF-α is one of the factors responsible for insulin resistance [33, 34], while increased interleukin-6 levels may also contribute to its development [35, 36]. Apart from a causative role in the pathogenesis of insulin resistance, increased monocyte production of TNF-α and interleukin-6 may be induced by impaired insulin receptor action, thus leading to the development of a vicious circle that may contribute to increased morbidity and mortality in older adults.

Increased monocyte production of TNF-α and interleukin-6 may contribute to elevated plasma levels of both cytokines, as observed previously by other authors in older adults [3]. The monocyte isolation procedure used in the study allowed us to obtain similar numbers of monocytes in both age populations. Therefore, the obtained results reflect differences in monocyte secretory function and cannot be explained by various numbers of these cells in peripheral blood taken from participants of our study.

Previously [18] we did not observe any differences in monocyte cytokine release between the effect of statin/fibrate combination therapy and the effect of a statin and a fibrate administered alone in type 2 diabetes patients with mixed dyslipidaemia treated with metformin. Although some participants of that study, fulfilling the criteria of mixed dyslipidaemia, were included in the present analysis there are some disparities between the results of the two studies. In the present study the strongest effect on TNF-α and interleukin-6 release was observed in older adults receiving the combination therapy, and the impact of this treatment option was stronger in older than in younger adults. These findings as well as the most pronounced effect on plasma lipids and on hsCRP suggest that older adults, particularly at high cardiovascular risk, may gain the greatest benefits from statin/fibrate combination therapy. In turn, in the population under the age of 50 years, the benefits statin/fibrate combination therapy may be less pronounced.

Interestingly, unlike TNF-α and interleukin-6, treatment-induced changes in interleukin-1β and MCP-1 release did not differ between patients belonging to both age populations, and were similar in extent in patients receiving statin/fibrate combination therapy and those receiving only one hypolipidaemic agent. This finding suggests that cellular signal pathways regulating monocyte secretion of various cytokines are partially distinct. Monocyte secretory function is modulated by many factors, influencing cytokine release both via direct and indirect mechanisms [37]. The net effect is a result of their mutual interactions, and this complex regulation seems to explain well the lack of parallel changes in the release of the investigated cytokines in our study. Interestingly, this explanation may also account for the finding that, contrary to TNF-α and interleukin-6, baseline release of interleukin-1β and MCP-1 did not depend on age.

The improvement in plasma lipids does not seem to play a role in reducing monocyte cytokine release by simvastatin, fenofibrate, and simvastatin/fenofibrate combination therapy in younger as well as in older adults with type 2 diabetes and atherogenic dyslipidaemia. However, the presence of correlations between the effects on cytokine release and on hsCRP shows that monocyte-suppressing effects of hypolipidaemic treatment options investigated in the present study contribute to their systemic anti-inflammatory effects, and partially explains the different hsCRP levels in both age populations.

Because this study was an observational one we can only hypothesise about the mechanisms responsible for the age-dependent effects of fenofibrate. They may result from changes in insulin sensitivity. In line with this hypothesis, the effect of simvastatin, having a neutral effect on glucose homeostasis, was unrelated to age. On the other hand, our findings may be explained by alterations in PPAR-α transmission. PPAR-α expression decreases with age, while PPAR-α downstream genes for some its key pathways, including nuclear factor-κB, activator protein-1 and signal transducers and activators of transcription, are up-regulated by aging [38]. Therefore, restoring normal PPAR-α signalling may be more important if this signalling is impaired, which takes place in older adults, than when intact.

This study has some limitations. The most important of them is a limited number of participants, as well as

a short treatment period, limiting the statistical significance of the findings. Secondly, our results should be interpreted with caution because the study investigated only surrogate endpoints. Moreover, our study was only a retrospective analysis and its findings should be confirmed in larger trials with hard endpoints. Finally, the study protocol does not allow us to answer the question of whether a similar relationship between the age and the strength of hypolipidaemic therapy is observed in patients not suffering from diabetes and not receiving metformin.

Conclusions

The results of the study indicate that the effect of fenofibrate and simvastatin/fenofibrate combination therapy on proinflammatory cytokine release and low-grade systemic inflammation is more pronounced in older than in younger adults. They indicate that age may partially determine monocyte-suppressing and systemic anti-inflammatory effects of fibrates, suggesting that older adults may benefit more from fibrate and statin/ /fibrate combination therapy.

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