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Atorvastatin and fenofibric acid differentially affect the release of adipokines in the visceral and subcutaneous cultures of adipocytes that were obtained from patients with and without mixed dyslipidemia

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Abstract:
In this study, we compared the effects of atorvastatin and fenofibric acid, which were administered alone or in combination, on the secretory function of human adipocytes that were obtained from the visceral and subcutaneous adipose tissues of 19 mixed dyslipidemic patients and 19 subjects with a normal lipid profile.
The adipocytes were incubated *in vitro* in the presence of atorvastatin and/or fenofibric acid. The secretory function of the cells was determined using ELISA assays.
The visceral adipocytes released significantly more adiponectin and IL-6 and less PAI-1 than those that were obtained from subcutaneous tissue. The levels and patterns of adipokine release differed between the patients with or without lipid abnormalities and between the adipocytes that were obtained from visceral or subcutaneous adipose tissue. The culture that contained hypolipidemic drugs resulted in the significant changes of the release of adipokines. The effects of atorvastatin and fenofibric acid on the hormonal function of human adipocytes may be, in part, responsible for the clinical efficacy of these drugs in the prevention and treatment of dyslipidemia-related cardiovascular and metabolic disorders. The study supports the concept that the pleiotropic effects of fenofibrate and atorvastatin may be, in part, a result of their impact on the secretory function of adipocytes.

Key words:
adipokines, adipocytes, preadipocytes, hypolipidemic agents, pleiotropic effects

Abbreviations: BrdU – 5-bromo-2'-deoxyuridine, CABG – coronary artery bypass grafting, DMEM – Dulbecco's modified Eagle's medium, ELISA – enzyme-linked immunosorbent assay, FBS – fetal bovine serum, HDL – high density lipoproteins, HEPES – 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, HMG-CoA – 3-hydroxy-3-methylglutaryl coenzyme A, HMW – high molecular weight, IBMX – isobutylmethylxanthine, IDF – International Diabetes Federation, IL – interleukin, LDL – low density lipoproteins, MS – metabolic syndrome, MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-

zolinum bromide, NCEP ATP III – National Cholesterol Education Program Adult Treatment Panel III, PAI-1 – plasminogen activator inhibitor 1, PPAR – peroxisome proliferator-activated receptors, PPARα – peroxisome proliferator-activated receptor α, TNFα – tumor necrosis factor α, WHR – waist-to-hip ratio

Introduction

Since the adipokines were discovered, only scarce information regarding the secretory function of human adipocytes exists. Many studies show the effects of hypolipidemic drugs on the serum adipocytokine concentration or the effects of these compounds on animal fat cells. Conflicting results from the existing studies may be a result of the methodological limitations. The assessment of adipokines in the plasma does not precisely reflect their release by different types of adipose tissue, while the hormonal function of the adipose tissue shows marked interspecies differences. For these reasons, it is still unclear whether or how hypolipidemic agents affect the hormonal functions of human adipocytes. Therefore, in our study, we have decided to compare the effects of two lipid-lowering drugs, atorvastatin and fenofibric acid, on the release of leptin, adiponectin, resistin, TNFα, interleukin 6 and plasminogen activator inhibitor-1 (PAI-1) in cultured human adipocytes.

We have also investigated whether the following are true: 1) an adipocyte tissue origin determines the effect of hypolipidemic agents on the adipokine release, 2) the effect on the adipocyte secretory function differs between adipocytes that are obtained from patients with mixed dyslipidemia and those with a normal lipid profile, 3) the combination treatment with atorvastatin and fenofibric acid affects the adipocyte hormonal function to a greater extent than when these drugs are administered alone.

Numerous multicenter randomized trials and smaller studies have estimated the effects of hypolipidemic drugs on the serum concentrations of adipokines.[31] The treatment with bezafibrate [32] or the administration of fenofibrate [9] reduced the leptin levels in dyslipidemic patients who had type 2 diabetes mellitus. The bezafibrate-treated subjects who were enrolled in the Bezafibrate Infarction Prevention study displayed higher adiponectin levels than the placebo-treated patients [18]. Fenofibrate, however, did not significantly alter the adiponectin and resistin concentrations in obese females who had type 2 diabetes mellitus [1] or the total adiponectin in hypertriglyceridemic patients [32]. Atorvastatin increased the plasma adiponectin levels in subjects who had a high cardiovascular risk [2], but it did not affect the plasma adiponectin, leptin and resistin levels in the dyslipidemic population [6, 7]. HMG-CoA inhibitors other than atorvastatin induced various effects on the serum concentrations of adipokines [3, 11–13, 15, 22, 23].

Even fewer studies have provided data regarding animal adipocyte culture and the release of adipokines. The results of these studies have provided us with only slightly more coherent results. Bezafibrate and fenofibrate significantly increased the adiponectin production in wild-type mice and 3T3-L1 adipocytes [18, 30]. In the visceral fat deposits of diabetic rats that were treated with fenofibrate, the expression of adiponectin was increased, while the expression of TNFα was reduced [5]. However, in other studies, neither fat adiponectin nor leptin expression was affected by the fibrate treatment in mice [16, 19, 29]. Moreover, fenofibrate paradoxically enhanced resistin expression in murine subcutaneous fat [16]. Contrary to the results with human plasma, statins increased the production of adiponectin and its mRNA expression in mouse 3T3-L1 adipocytes [39] and in the white adipose tissue of rats that spontaneously developed type 2 diabetes mellitus [4].

Only a few researchers have published the results of hypolipidemic drugs on human fat tissue culture. Previously, we showed that atorvastatin and fenofibric acid are potent drugs that modify the release of cytokines in human fat tissue explants [25]. Fenofibric acid possesses agonist activity toward PPARα; however, contrary to the fenofibrate esters, it lacks the ability to block the liver X receptor. Therefore, the *in vivo* effects of fenofibrate cannot solely be attributed to PPARα stimulation [37]. Laumen et al. have shown that a potent HMG-CoA reductase inhibitor significantly reduces the secretion of PAI-1 in adipocyte culture [26].

Materials and Methods

Subjects

The abdominal subcutaneous and visceral (omental) adipose tissue samples were obtained from 19 untreated patients who were newly diagnosed with mixed

dyslipidemia and 19 subjects who displayed a normal lipid profile (35–65 years old) who were undergoing laparoscopic abdominal or gynecological surgery. Mixed dyslipidemia was diagnosed in the patients who met the following criteria: plasma total cholesterol > 200 mg/dl, LDL cholesterol > 130 mg/dl and triglycerides > 200 mg/dl. All of the patients were fasted overnight before the tissue was removed. Subjects who displayed the symptoms or systemic signs of inflammation, cancer, autoimmune disorder and any other serious acute or chronic disorder, or those who were taking hypolipidemic agents or medications that could influence the adipose tissue metabolism, interact with statins and fibrates, or produce anti-inflammatory effects (including glucocorticosteroids, non-steroidal anti-inflammatory drugs and angiotensin-converting enzyme inhibitors) were excluded from the study. The study was performed according to the Declaration of Helsinki after it was accepted by the local ethics committee. All of the patients provided written informed consent. The samples were transported to the laboratory in a sterile container within 30 min of their removal.

Reagents

Atorvastatin, fenofibric acid, mitomycin C, trypan blue, MTT, BrdU and Red Blood Cell Lysing Buffer were purchased from Sigma Chemicals (St. Louis, MO, USA). Preadipocyte Basal Medium, Preadipocyte Growth Medium, PBS, HEPES, collagenase (Type 1), FBS, albumin, L-glutamine, penicillin, streptomycin, insulin, dexamethasone, IBMX and the AdipoRed Assay Reagent were obtained from Cambrex Bioscience (Walkersville, MD, USA). The ELISA kits that were used for the quantification of adiponectin, leptin, resistin, TNFα, interleukin 6 and PAI-1 were purchased from R&D Systems Inc. (Minneapolis, MN, USA). The commercial kits that were used for the assessment of the lipid profiles were acquired from BioMerieux (Marcy-l'Etoile, France).

Preadipocyte cultures

Human subcutaneous and visceral preadipocytes were isolated and cultured according to the methods that were previously described [8, 40] with minor modifications. The fragments of the entire adipose tissue that was isolated from the patients (100 mg/patient) were cut into small pieces of less than 10 mg each. The explants of adipose tissue were rinsed in 10 ml of PBS that contained albumin (3 ml/g of tissue) for about 5 min (on an orbital shaker) to reduce any contamination of the tissue with blood. Next, the tissue fragments were centrifuged at 400 × g for 30 s to separate the excess of blood and pieces of tissues that contained insufficient fragments;. The floating adipose tissue that was separated from the medium was digested (collagenase type 1, pH 7.4) and separated from the tissue aggregates by filtration (200 and 70 μm cell strainer). The filtered cells were suspended in 15 ml of Preadipocyte Basal Medium and were centrifuged to separate the mature adipocytes and the extracellular fat from the stromal and vascular cells.

To remove the red blood cells, the resulting sediment was resuspended for 2 min in Red Blood Cell Lysing Buffer at room temperature. After this process, the cells were filtered and resuspended in 5 ml of Preadipocyte Basal Medium. The preadipocytes and fibroblasts were exhibited on mitomycin C (4 μg/ml), which is the established chemotherapeutic that, *in vitro*, inhibits the proliferation of the cells that are characterized by a high mitotic index [24]. The preadipocytes that were grown in Preadipocyte Basal Medium without growth supplements revealed a low mitotic index, which was confirmed by BrdU incorporation.

The adhered preadipocytes (in majority) were scraped and resuspended in a medium that contained the growth supplements that were needed to induce the growth and differentiation of the preadipocytes into mature adipocytes. The number of cells was counted using an optical microscope, and the preadipocyte concentration was adjusted to 1×10^5 cells per 35 mm Petri dish. The Preadipocyte Growth Medium was changed every 2 days. Lipid vacuoles began to appear at 3–4 days after the beginning of the induction, and they continued to increase in number and size until days 7 to 9.

The induced preadipocytes and mature adipocytes were stained with AdipoRed Assay Reagent (Cambrex Bioscience, MD, USA). More than 95% of the cultured cells reacted with AdipoRed. The rest was composed of non-induced cells (fibroblasts and endothelial cells). No erythrocytes or leukocytes were detected as confirmed by the standard histological method. On day 8, the Preadipocyte Growth Medium was removed and replaced with Basal Medium alone (this medium served as a control) or with atorvastatin (0.1, 1.0 or 2.0 μM) and fenofibric acid (0.25, 2.5 or 5.0 μM) (Sigma Chemicals, St. Louis, MO, USA).

Fenofibric acid was used instead of fenofibrate because it is a form of this drug that is biologically active and easily dissolved in water [20]. The incubation was continued for the following 24 h. Twenty-four hours was chosen as the maximal incubation time based on a published study; in this study by other authors, after 24 h, the adipose cells were still viable and responsive, and the secretion activity was maximal. To be sure that the effects of the drugs that were employed in this study resulted from their regulatory action and not from their toxicity, we assayed the cell viability with the 0.4% Trypan Blue Exclusion Test and MTT conversion (Sigma-Aldrich, St. Louis, MO, USA). More than 95% of the cultured cells were still viable and responsive. At the end of the incubation, the medium was collected and frozen at –70°C until it was assayed for the studied markers. Each group of experiments was repeated in 3 independent experiments.

Tab. 1. Baseline characteristics of patients

	Patients with normal lipid profile	Patients with mixed dyslipidemia
Number of patients	19	19
Age (years)	48.6 ± 2.3	49.3 ± 2.4
Men/Women	10/9	10/9
Smokers	8 (42.1%)	7 (36.8%)
Body mass index (kg/m^2)	28.5 ± 1.6	28.9 ± 1.9
WHR – women	0.88 ± 0.03	0.9 ± 0.03
WHR – men	1.01 ± 0.04	1.05 ± 0.05
Type 2 diabetes	2 (10.5%)	3 (15.8%)
Stable coronary artery disease	3 (15.8%)	4 (21.1%)
Stable cerebrovascular disease	1 (5.3%)	2 (10.5%)
Previous atherosclerotic clinical events[1]	1 (5.3%)	0 (0%)
Fasting glucose (mg/dl)	96.2 ± 3.4	95.9 ± 3.1
HOMA	4.9 ± 0.6	5.0 ± 0.8
HbA_{1c} (%)	5.4 ± 0.5	5.6 ± 0.6
Total cholesterol (mg/dl)	178.0 ± 6.2	264.3 ± 10.2*
LDL cholesterol (mg/dl)	113.1 ± 5.0	159.1 ± 7.5*
HDL cholesterol (mg/dl)	46.9 ± 2.2	40.9.0 ± 2.4*
Triglycerides (mg/dl)	152.4 ± 15.2	275.0 ± 18.2*

[1]Unstable coronary artery disease, myocardial infarction, stroke, transient ischemic attack or intermittent claudication if the occurrence was more than 6 months before the beginning of the study. The data represent the mean ± SD. * $p < 0.001$ *vs.* the patients with a normal lipid profile

Laboratory assays

The plasma levels of total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides were assessed colorimetrically 12 h after the last meal on the day that preceded the operation.

The adipocyte release of adiponectin, leptin, resistin, TNFα, interleukin 6 and PAI-1 was determined by ELISA according to the manufacturer's instructions within 48 h after the collection.

Statistical analysis

All of the data are expressed as the mean ± SD. First, the distribution of the variables was analyzed. The outcomes for adiponectin, leptin, resistin, TNFα, interleukin-6 and PAI-1 were natural-log transformed to satisfy the assumptions of normality and equal variance. Parametric tests were used for the statistical analysis because, after the logarithmic transformation, the obtained values were normally distributed. The comparisons between the groups were performed using a one-way ANOVA that was followed by the *post-hoc* Bonferroni test; p values of less than 0.05 were considered to be statistically significant. All of the calculations were made using the GraphPad Prism 2.01 software and Statistica 6.1.

Results

Baseline characteristics

There were no significant differences in the age, weight, WHR and sex between the patients with and without mixed dyslipidemia (Tab. 1). Of the patients, 25–31% (according to NCEP ATP III) or 50–67% (according to IDF) met the diagnosis criteria of the metabolic syndrome. Both groups were also comparable with respect to their medical background and clinical and laboratory characteristics. However, there were important differences in the secretory functions between the adipocytes of the patients with and without mixed dyslipidemia and between the adipocytes of the visceral and subcutaneous adipose tissue (Tab. 2). The adipocytes that were obtained from the visceral and the subcutaneous adipose tissue of the patients with mixed dyslipidemia released greater amounts of leptin, resistin, TNFα, interleukin-6 and PAI-1 and re-

Tab. 2. Baseline adipokine release by human adipocytes

Adipokine	Patients with a normal lipid profile		Patients with mixed dyslipidemia	
	Visceral adipocytes	Subcutaneous adipocytes	Visceral adipocytes	Subcutaneous adipocytes
Adiponectin (ng/ml)	29.8 ± 3.6	19.0 ± 2.8†	19.5 ± 4.0§	15.0 ± 2.0*‡
Leptin (pg/ml)	422.8 ± 30.8	402.6 ± 38.4	756.8 ± 16.4$^{\|\|}$	629.8 ± 23.6$^{\|\|}$
Resistin (pg/ml)	105.0 ± 23.2	100.2 ± 25.0	215.3 ± 30.1$^{\|\|}$	193.2 ± 32.0$^{\|\|}$
TNFα (pg/ml)	55.0 ± 7.4	59.5 ± 8.4	181.1 ± 14.8$^{\|\|}$	199.9 ± 12.0$^{\|\|}$
Interleukin-6 (pg/ml)	219.3 ± 15.2	150.0 ± 30.8†	319.0 ± 22.4$^{\|\|}$	242.5 ± 11.6*$^{\|\|}$
PAI-1 (ng/ml)	152.1 ± 8.8	203.8 ± 10.8*	204.0 ± 18.0‡	247.5 ± 9.2*‡

Data represent the mean ± SD. * $p < 0.05$, † $p < 0.01$ *vs.* the visceral adipocytes from the same group of patients. ‡ $p < 0.05$; § $p < 0.01$, $^{\|\|}$ $p < 0.001$ *vs.* the respective adipocytes of the subjects with a normal lipid profile

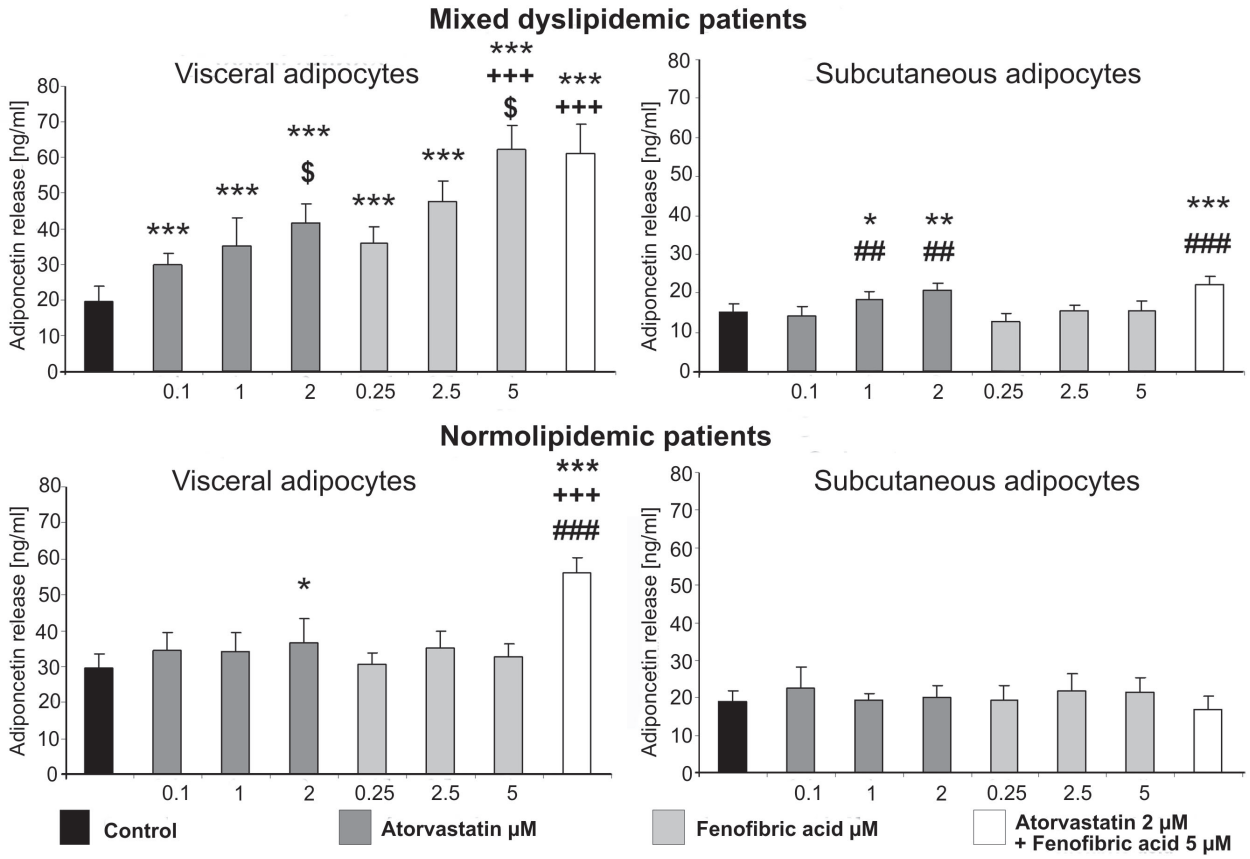

Fig. 1. Effect of atorvastatin and fenofibric acid on adiponectin release by the adipocytes of the patients with mixed dyslipidemia and the subjects with a normal lipid profile. The data represent the mean ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* the untreated adipocytes, $^{+++}$ $p < 0.001$ *vs.* the adipocytes that were treated with 2 μM of atorvastatin; $^{\#\#}$ $p < 0.01$, $^{\#\#\#}$ $p < 0.001$ *vs.* the adipocytes that were treated with 5 μM of fenofibric acid; $^{\$}$ a dose-dependent effect of the drug

duced amounts of adiponectin than the adipocytes that were obtained from the same fat deposits of the patients with a normal lipid profile. In both groups of patients, who had either mixed dyslipidemia or a normal lipid profile, the omental adipocytes released significantly more adiponectin and interleukin-6 and less PAI-1 than the adipocytes that were obtained from the subcutaneous tissue.

Adiponectin (Fig. 1)

In the omental adipocytes of the patients with mixed dyslipidemia, both drugs increased the release of adiponectin in a dose-dependent manner. The increase in the adiponectin release was 53.2–114.1% ($p < 0.001$) for atorvastatin and 83.3–218.6% ($p < 0.001$) for fenofibric acid. The effect of fenofibric acid was stronger than that of atorvastatin ($p < 0.001$). When they were administered together, both drugs increased adiponectin release by 213.5% ($p < 0.001$), and this effect was comparable to that induced by fenofibric acid alone. In the adipocytes of the subcutaneous adipose tissue that was derived from the mixed dyslipidemic patients, the 1 and 2 μM doses of atorvastatin increased the adiponectin release by 23.3% ($p < 0.05$) and 39.2% ($p < 0.01$), respectively. At any concentration, fenofibric acid did not significantly affect the release of this adipokine. The combination treatment did not provide any benefit over the administration of fenofibric acid alone. In the omental adipocytes of the patients with a normal lipid profile, the highest dose of atorvastatin increased the adiponectin secretion by 31.3% ($p < 0.05$), while the remaining doses of atorvastatin and 2.5 μM of fenofibric acid did not significantly affect the release of this adipokine. In the cultures that were treated with both of these agents, the adiponectin release increased by 88.7% ($p < 0.001$), and this effect was more pronounced than when these drugs were administered alone ($p < 0.001$). Neither the administration of atorvastatin nor fenofibric acid

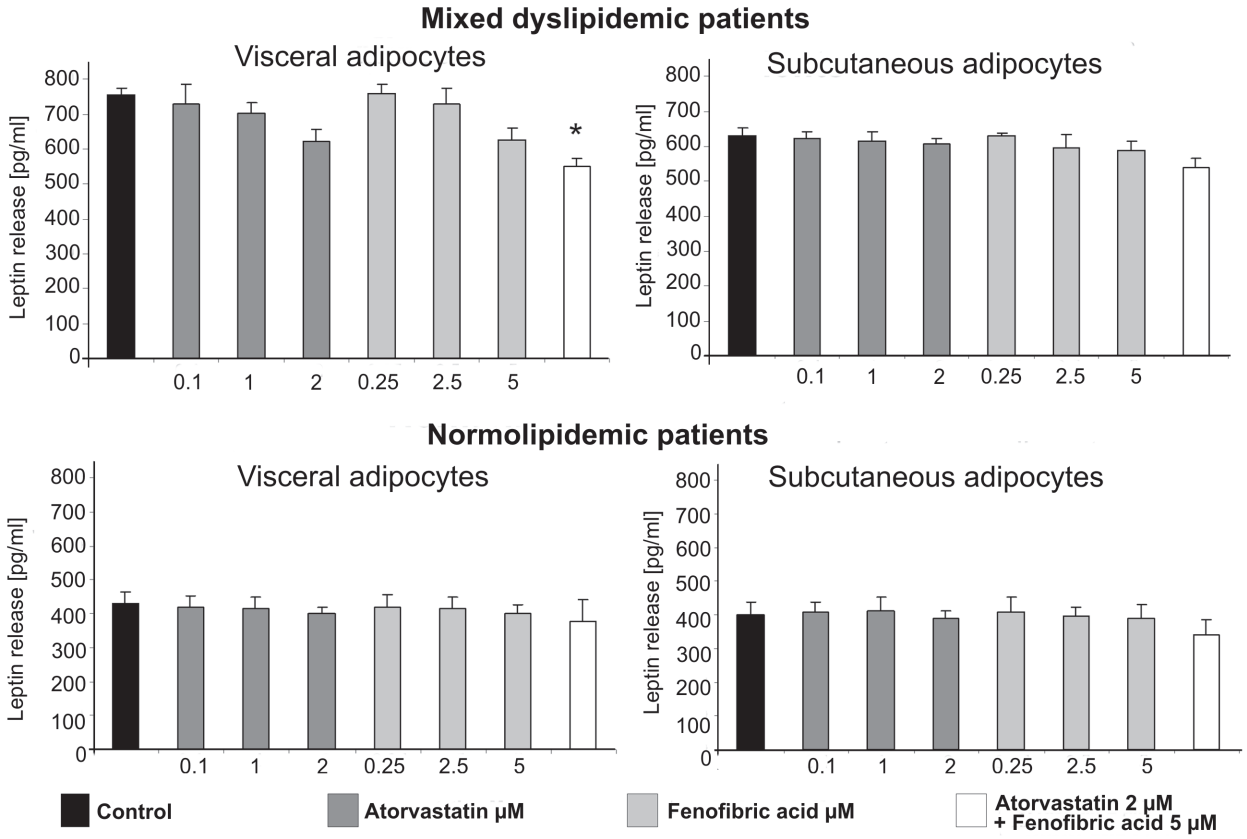

Fig. 2. Effect of atorvastatin and fenofibric acid on leptin release by the adipocytes of the patients with mixed dyslipidemia and the subjects with a normal lipid profile. The data represent the mean ± SD. * $p < 0.05$ *vs.* the untreated adipocytes

altered the adiponectin release by the adipocytes of the subcutaneous tissue in the patients who lacked lipid abnormalities.

Leptin (Fig. 2)

In the visceral adipocytes of the patients with mixed dyslipidemia, neither 2 μM of atorvastatin nor 5 μM of fenofibric acid significantly affected the release of leptin. However, the combined treatment of these compounds significantly reduced leptin release (by 27.2%, $p < 0.05$). In the adipocytes that were obtained from the subcutaneous tissue of the mixed dyslipidemic patients, neither of the drugs nor their combination significantly affected leptin release. In the adipocytes that were obtained from the patients without lipid abnormalities, irrespective of the site of the tissue origin, neither of the drugs nor their combination significantly affected leptin release.

Resistin (Fig. 3)

At their highest concentrations, both fenofibric acid and atorvastatin significantly reduced (by 35.5%, $p < 0.01$ and by 35.6%, $p < 0.01$, respectively) resistin release by the omental adipocytes of the dyslipidemic patients. The combined treatment of these drugs achieved the strongest reduction in resistin release by the visceral adipocytes of the dyslipidemic patients (a reduction by 54.8%, $p < 0.001$), and this reduction was superior to the effects when each drug was administered alone ($p < 0.01$). In the adipocytes of the subcutaneous tissue that was obtained from the dyslipidemic patients, only the combined treatment with both agents reduced resistin release (by 32.5%, $p < 0.01$). In the adipocytes of the patients with a normal lipid profile, neither atorvastatin nor fenofibric acid changed the secretion of resistin; this result was unaffected by the site of the tissue isolation. A com-

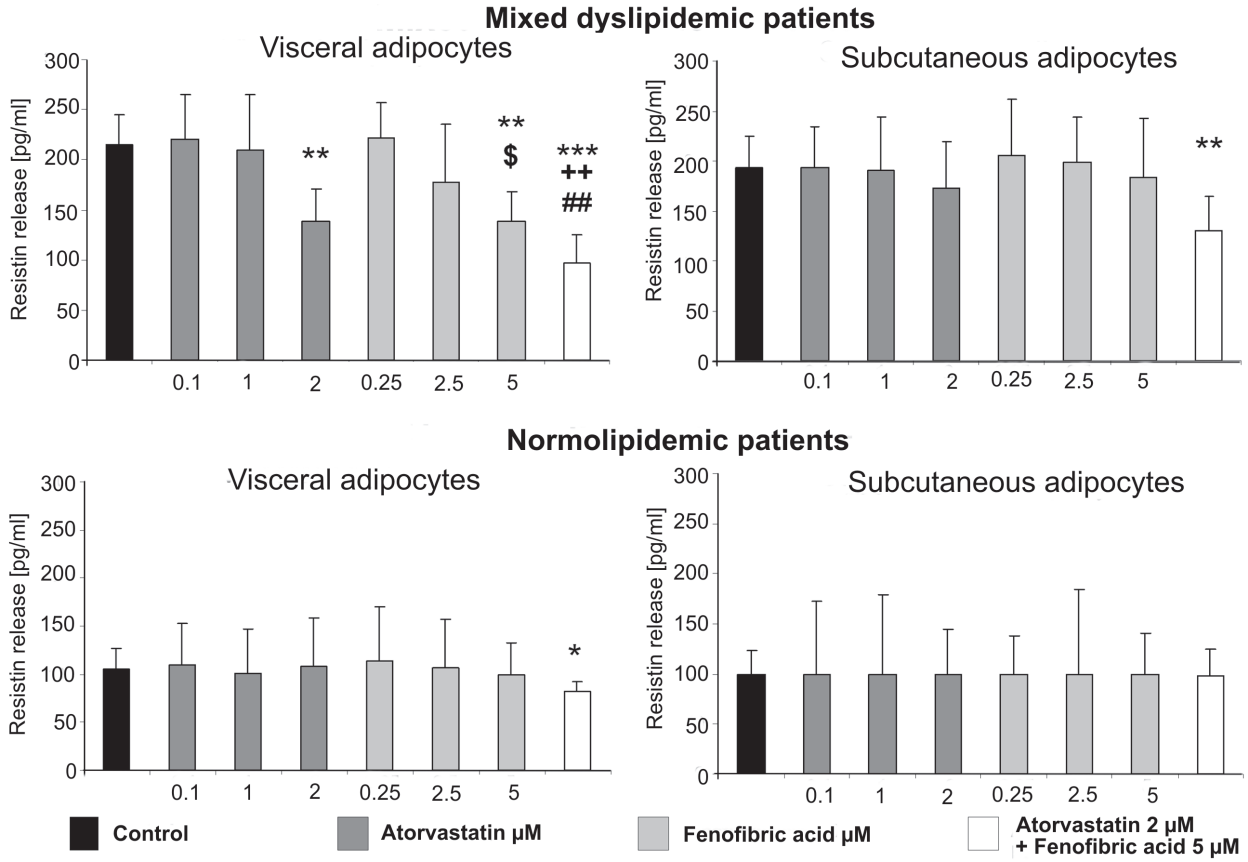

Fig. 3. Effect of atorvastatin and fenofibric acid on resistin release by the adipocytes of the patients with mixed dyslipidemia and the subjects with a normal lipid profile. The data represent the mean ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* the untreated adipocytes; $^{++}$ $p < 0.01$ *vs.* the adipocytes that were treated with 2 μM of atorvastatin; $^{\#\#}$ $p < 0.01$ *vs.* the adipocytes that were treated with 5 μM of fenofibric acid; $^{\$}$ a dose-dependent effect of the drug

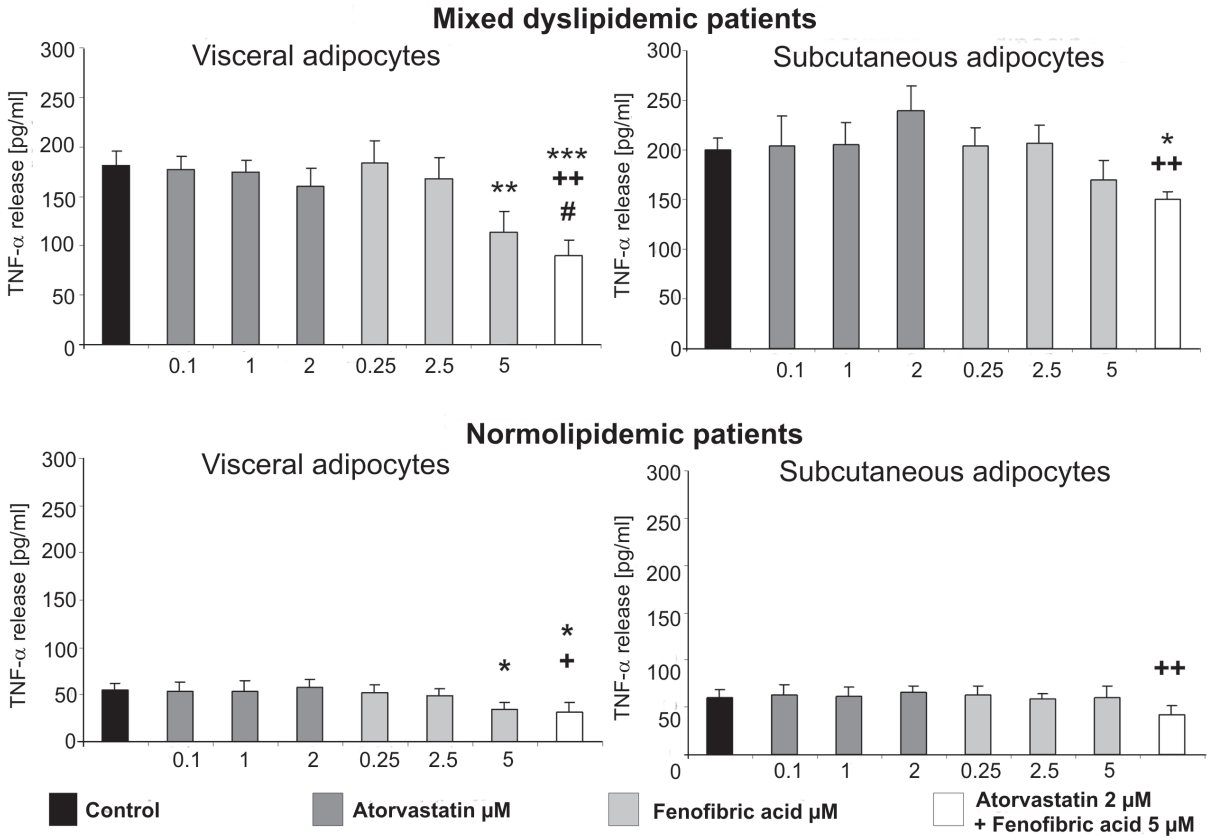

Fig. 4. Effect of atorvastatin and fenofibric acid on TNFα release by the adipocytes of the patients with mixed dyslipidemia and the subjects with a normal lipid profile. The data represent the mean ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* the untreated adipocytes; $^{+}$ $p < 0.05$, $^{++}$ $p < 0.01$ *vs.* the adipocytes that were treated with 2 μM of atorvastatin; $^{\#}$ $p < 0.05$ *vs.* the adipocytes that were treated with 5 μM of fenofibric acid

bined treatment with both of these agents reduced resistin release in the adipocytes of the omental fat (by 21.7%, $p < 0.05$) but not of the subcutaneous tissue.

TNFα (Fig. 4)

Only the 5 μM dose of fenofibric acid reduced the release of TNFα from the visceral adipocytes of the mixed dyslipidemic patients by 37.2% ($p < 0.01$). At any concentration, atorvastatin did not significantly affect the release of TNFα from the visceral adipocytes of the mixed dyslipidemic patients. When they were administered together, fenofibric acid and atorvastatin diminished the TNFα secretion by these cells by 50.0% ($p < 0.001$), and this effect was more profound than that of fenofibric acid ($p < 0.05$) when it was administered alone. Neither fenofibric acid nor atorvastatin significantly changed the TNFα release by the adipocytes that were obtained from the subcutaneous tissue of the patients with mixed dyslipidemia, and this result was not affected by the concentrations of the drugs. When both of the agents were administered together, TNFα secretion decreased by 24.7% ($p < 0.05$). In the omental adipocytes of the patients with a normal lipid profile, only 5 μM of fenofibric acid reduced the TNFα release by 22.3% ($p < 0.05$), while the combined treatment decreased it by 26.1% ($p < 0.05$). The effect of the combined treatment was stronger than the effect of atorvastatin ($p < 0.05$). In the adipocytes of subcutaneous origin from the normolipidemic patients, neither of the drugs nor their combination significantly affected TNFα release.

Interleukin-6 (Fig. 5)

In the visceral adipocytes of the mixed dyslipidemic patients, only the combination of fenofibric acid and atorvastatin reduced the secretion of interleukin-6 by 28.6% ($p < 0.05$). In the adipocytes of the subcutaneous tissue that was obtained from the dyslipidemic patients, only the combination of fenofibric acid and

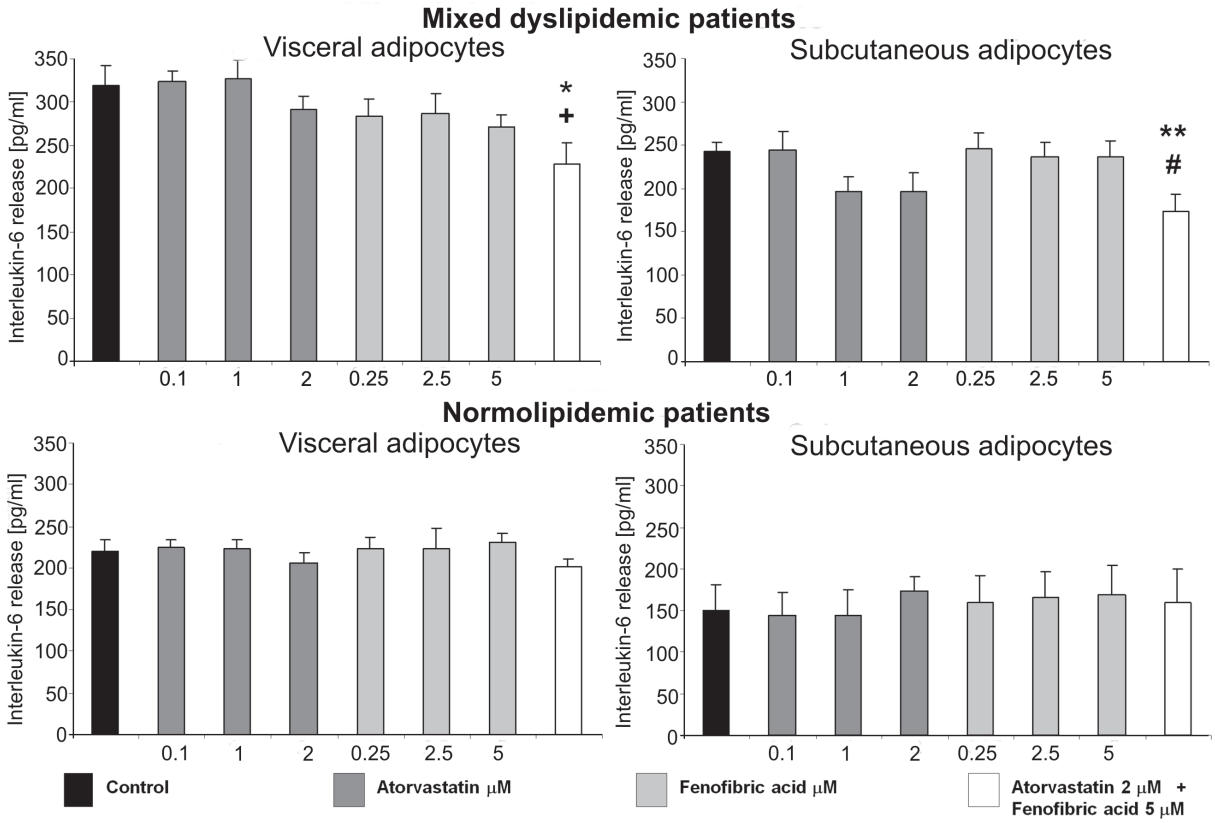

Fig. 5. Effect of atorvastatin and fenofibric acid on interleukin-6 release by adipocytes of the patients with mixed dyslipidemia and the subjects with a normal lipid profile. The data represent the mean ± SD. * $p < 0.05$, ** $p < 0.01$ *vs.* the untreated adipocytes; $^{+}$ $p < 0.05$ *vs.* the adipocytes that were treated with 2 μM of atorvastatin; $^{\#}$ $p < 0.05$ *vs.* the adipocytes that were treated with 5 μM of fenofibric acid

atorvastatin reduced the secretion of interleukin-6 by 28.6% ($p < 0.01$). In the patients who lacked lipid abnormalities, neither of the drugs nor their combination altered interleukin-6 secretion by the adipocytes, and this result was independent of the site of the tissue origin.

PAI-1 (data not shown)

No significant changes in PAI-1 release by the omental adipocytes and by the adipocytes of the subcutaneous adipose tissue were observed in either the mixed dyslipidemic or the normolipidemic patients.

Discussion

The major finding of this study is that atorvastatin and fenofibric acid exhibit a moderate, but similar in extent, effect on the secretory function of human adipocytes; this represents one of the pleiotropic actions of the HMG-CoA reductase inhibitors and the PPARα activators.

Our study has revealed that the adipocytes of the patients with mixed dyslipidemia released significantly more leptin, resistin, TNFα, interleukin-6 and PAI-1 and significantly less adiponectin than the adipocytes of the subjects with a normal lipid profile. Strict inclusion criteria and the similar clinical and laboratory characteristics of the patients with and without mixed dyslipidemia minimized the possibility that these differences resulted from the impact of other concurrent diseases or concomitant therapies. Due to the anti-atherogenic, anti-inflammatory and metabolically beneficial actions of adiponectin and the opposing effects of the remaining markers, our findings seem to be clinically relevant. Our results suggest that an abnormal adipocyte adipokine release may contribute to the earlier development and accelerated progression of atherosclerosis and metabolic syndrome in the patients with mixed dyslipidemia.

In accordance with our expectations, the omental adipocytes secreted higher levels of interleukin-6, but they unexpectedly secreted less PAI-1. It is possible that other cells within the adipose tissue that interact with the adipocytes may affect the capacity of the adipocytes to release these markers [25]. Our study also revealed that the visceral adipocytes produce more adiponectin. A locally increased production of this adipokine may constitute a compensatory mechanism that prevents the development of metabolic and cardiovascular complications in response to an increase in the visceral fat mass [27]. Interestingly, the differences in the secretion of adiponectin, interleukin-6 and PAI-1 from both types of adipocytes were not only identified in dyslipidemic patients, but they were also observed in those patients who displayed a normal lipid profile. Although the adipocytes of the dyslipidemic patients secreted altered levels of all of the studied markers, the relative contribution of the visceral and subcutaneous adipocytes in adipokine release was similar in the subjects with and without lipid abnormalities. From an anatomic standpoint, the products of the visceral and subcutaneous fat supply the liver and skeletal muscles, respectively.

We have shown that both hypolipidemic agents moderately altered the secretory function of human adipocytes. In our study, both atorvastatin and fenofibric acid were used at concentrations that may be achieved in the plasma and peripheral tissues (including adipocytes) during a treatment with those agents [10, 28]; additionally, they were tested at concentrations that were higher than those that could be observed in the plasma and tissues of atorvastatin- and fenofibrate-treated patients. In *in vitro* studies, supraphysiological doses are often required to produce an effect because in clinical trials, statins and fibrates are administered for a period of weeks or even months, while the cell cultures are treated with these drugs for only hours. It should be stressed that the supraphysiological concentrations at which atorvastatin and fenofibric acid were administered in this experiment are in the same range as the concentrations that are used in other tissue culture studies [14, 35]. This suggests that the obtained results are of clinical significance. For PAI-1, our results are in accordance with those of He et al. [17], who did not observe any effects of fenofibrate or other PPARα activators on PAI-1 production in human adipose tissue; however, our results are in contrast with the results of Laumen et al. [26], in which rosuvastatin inhibited PAI-1 mRNA expression and secretion in the cultures of human adipocytes in a concentration-dependent manner. Additionally, Khan et al. observed that the pleiotropic effects of statin affected the secretion of adipokines in the mouse model [21]. They aimed to explain the reason for the unchanged total adiponectin level despite the lowered high molecular weight fraction. An impairment of the secretion function in the adipocytes has been observed. Therefore, it cannot be ruled out that the different HMG-CoA reductase inhibitors exhibit various effects on the adipocyte secretion of this marker in humans.

When interpreting *in vitro* studies, one should remember that study-dependent differences in the effects of hypolipidemic agents on adipokine production and release may partially result from the fact that the adipose tissue samples were obtained from various regions of the body. To the best of our knowledge, only one previous study compared the effects of a lipid-lowering drug on the hormonal function of visceral and subcutaneous adipose tissue. Saito et al. [36] observed that adiponectin gene expression was significantly increased by pravastatin in patients with coronary artery disease. In our study, the atorvastatin- and fenofibric acid-induced changes in adipokine secretion were more markedly expressed in the visceral adipocytes. This difference was particularly noticeable for adiponectin and was generally more pronounced for the adipocytes of dyslipidemic patients. Therefore, our results seem to indicate that the tissue origin of the adipocyte may, to some degree, determine the action of lipid-lowering drugs on adipokine release.

Only a few studies have investigated which of the two major groups of hypolipidemic agents more strongly affects the hormonal function of adipose tissue or whether a combined treatment with statins and fibrates may offer any additional benefits, and the results of these studies were contradictory. In the study by Otto et al. [34], neither the 6-week administration of atorvastatin nor fenofibrate significantly affected the plasma levels of adiponectin and resistin in patients with type 2 diabetes and mixed hyperlipoproteinemia. Yin et al. [38] observed that an 8-week treatment of dyslipidemic type 2 diabetic patients with both fenofibrate and rosiglitazone increased the plasma adiponectin levels and decreased the plasma resistin levels; however, this result was not observed when the patients were treated with both atorvastatin and glibenclamide. In the study by Koh et al. [22], the treatment of mixed

dyslipidemia with both atorvastatin and fenofibrate or with fenofibrate alone reduced the plasma adiponectin levels to a greater extent than with the administration of atorvastatin alone. Discrepancies between the results of all of these studies may result from their inherent limitations. At most, two adipokines were measured in each of these studies, and adipokines were assessed only in the plasma. Furthermore, the results of the study by Yin et al. [38] may be confounded by their simultaneous use of antidiabetic agents (rosiglitazone or glibenclamide). To the best of our knowledge, our study is the first one that compared the effects of HMG-CoA reductase inhibitors and PPARα activators on the level of secretory function of adipocytes. For most of the studied markers, the magnitude of the drug-induced changes in the release was similar for the adipocytes of the mixed dyslipidemic patients who were treated with atorvastatin and fenofibric acid. In the adipocytes of the visceral tissue, however, fenofibric acid was superior to atorvastatin in stimulating adiponectin secretion, and only the highest dose of fenofibric acid reduced TNFα release; this release was not observed with atorvastatin. In turn, in the adipocytes of the subcutaneous adipose tissue, atorvastatin was the only drug that enhanced adiponectin secretion. This may suggest that although both statins and fibrates can alter the function of the adipocytes regardless of the place of their origin, the visceral adipocytes may be a more important target for the fibrates, whereas the subcutaneous tissues are the more important target for the statins. Based on the observed effects of both of the agents, it seems, however, that neither atorvastatin nor fenofibrate can be recommended as a superior treatment for the secretory function of human adipocytes.

Recently, Haluzik et al. [16] have shown that fenofibrate increased the plasma adiponectin levels and the muscle expression levels of adiponectin mRNA in mice that were fed with normal chow but not in mice that received lipogenic food. This may suggest that the action of lipid-lowering agents on adipokine production and release depends on the baseline levels of lipids. Although our results support the existence of a relationship between the baseline metabolic profile and the potency of drugs to alter adipokine secretion, they provided opposite results because the action of fenofibric acid and atorvastatin on the release of the assessed markers was stronger in the dyslipidemic subjects than in the subjects without lipid abnormalities. This may indicate that the pleiotropic effects of lipid-lowering agents at the level of human adipocytes are more pronounced in patients with a disturbed lipid metabolism than in the normolipidemic patients.

We have found, however, that especially when they were administered in combination, fenofibric acid and atorvastatin slightly affected the secretory function of human adipocytes that were derived from the group of normolipidemic patients. Although this result was less pronounced than for the adipocytes of the mixed dyslipidemic patients, this effect was significant for TNFα when the cultures were treated with the highest dose of fenofibric acid; it was significant for adiponectin in the adipocytes that were exposed to the highest dose of atorvastatin; and it was significant for adiponectin, resistin and TNFα when the adipocytes were exposed to both of these agents. Therefore, our study suggests that atorvastatin and fenofibrate that are administered at high doses may produce some benefits even in high-risk patients who lack lipid abnormalities.

Our study has some limitations. It is possible that the effect of atorvastatin and fenofibrate on adiponectin release may be even stronger than we observed because we assessed only the total adiponectin release. In the study by Oki et al. [33], fenofibrate that was administered to hypertriglyceridemic patients increased the serum high-molecular-weight adiponectin but not the total protein concentration. However, these observations should be treated with some caution because in the study of other authors [15], simvastatin affected the serum levels of neither the total nor the HMW adiponectin in healthy adult volunteers. In our study, the adipose tissue samples were collected under general anesthesia; therefore, we cannot exclude that the drugs that were used during the operative procedure may have modified the effects of atorvastatin and fenofibric acid on the studied markers. The impact of anesthesia on the measured parameters should not be discernible for several reasons: the adipocytes were cultured, the culture procedure that was used, which is described in the Materials and Methods section, is a timely procedure, the drugs that were administered by the anesthesia team have short half-elimination times, and the fat compartment is relatively poorly perfused. Moreover, to the best of our knowledge, there are no published data supporting the impact of anesthetics on the hormonal function of human adipocytes. Although the cell cultures were composed of more than 90% adipocytes, the confounding

effect of the secretion of the remaining cells cannot be excluded (e.g., resistin secretion). Finally, because we only included patients with mixed dyslipidemia, and other forms of lipid abnormalities were regarded as exclusion criteria, the question regarding the effects of hypolipidemic agents on the secretory function of human adipocytes in the remaining types of dyslipidemia requires further study.

In summary, our study has shown that the adipokine release levels and patterns in mixed dyslipidemic patients are disturbed in the adipocytes of both visceral and subcutaneous fat tissue. The study also revealed that atorvastatin and fenofibric acid produce their pleiotropic effect, in part, by changing the adipokine release by human adipocytes regardless of the site of their origin. This effect, which is more pronounced in the patients with mixed dyslipidemia and stronger when both of the drugs are administered together, may contribute to the clinical effectiveness of HMG-CoA reductase inhibitors and PPARα activators in the prevention and treatment of dyslipidemia-related disorders.

Declaration of interests
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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