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Original research article

# The effect of ezetimibe on adipose tissue hormones in patients with isolated hypercholesterolemia

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ABSTRACT

*Background:* Extra-lipid effects of ezetimibe, a new lipid-lowering agent, are so far poorly understood.
*Methods:* Twenty-two patients with elevated total and LDL cholesterol levels, statin-intolerant or having contraindications to statin therapy, were treated with ezetimibe (10 mg daily) for 90 days. Plasma levels of lipids, apolipoproteins, glucose homeostasis markers, leptin, adiponectin, visfatin, tumor necrosis factor-α (TNF-α), free fatty acids (FFA) and high sensitivity C-reactive protein (hsCRP) were examined at the beginning of the study and after 30 and 90 days of treatment.
*Results:* Compared with the control age-, sex-, and weight-matched healthy subjects, isolated hypercholesterolemic patients exhibited higher plasma levels of leptin, visfatin and TNF-α and lower plasma levels of adiponectin. Their baseline FFA and hsCRP levels were also increased. Ezetimibe decreased circulating levels of total cholesterol, LDL cholesterol and apolipoprotein B-100. The drug significantly reduced plasma levels of visfatin and only tended to reduce plasma levels of leptin, TNF-α, visfatin, FFA and CRP. The effect of ezetimibe on these markers was lipid-independent but stronger in insulin-sensitive than in insulin-resistant patients.
*Conclusions:* The obtained results indicate that the presence of isolated hypercholesterolemia is associated with abnormal hormonal function of the adipose tissue. They also show that ezetimibe induces relatively small changes in adipose tissue hormonal function and systemic inflammation in patients with elevated cholesterol levels.


## Introduction

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, have proved to be effective in the primary and secondary prevention of cardiovascular diseases [1,2]. It has been established that the benefits of statin therapy in cardiovascular diseases can be explained not only by the lipid-lowering potential of statins but also by nonlipid-related mechanisms (so-called "pleiotropic effects") [3]. Although HMG-CoA reductase inhibitors (statins) are mainstay drugs for management of elevated cholesterol levels, in some patients their administration is either insufficient or not tolerated. These patients may benefit from the treatment with ezetimibe, which, by blocking the NPC1L1 protein in the jejunal brush border, inhibits intestinal cholesterol absorption, reducing total and LDL cholesterol levels [4]. However, unlike statins, larger clinical trials assessing efficacy of ezetimibe provided contrasting results. In some studies, ezetimibe administered together with statins reduced the carotid intima-media thickness in patients at high cardiovascular risk [5] and in patients with type 2 diabetes and no prior cardiovascular events [6]. However, in other trials, ezetimibe did not result in a regression of carotid artery atherosclerosis in patients with familial hypercholesterolemia [7] and in patients with coronary artery disease or its equivalent [8], as well as it did not reduce the composite outcome of combined aortic-valve events and ischemic events in patients with aortic stenosis [9].

Unfortunately, in all these studies ezetimibe was administered together with a statin and this combination therapy was compared with a statin alone, statin–niacin combination, or with placebo. Therefore, the question whether ezetimibe administered alone is an effective anti-atherosclerotic drug still remains open. Because atherosclerosis is a complex process, which cannot be attributed to only one factor, hypolipidemic drugs producing non-lipid related

*Abbreviations:* CRP, C-reactive protein; FFA, free fatty acids; 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; TNF-α, tumor necrosis factor-α.

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effects are probably more efficient anti-atherogenic agents than those affecting only plasma lipids [1,2,10]. In this prospective study, we have investigated whether monotherapy with ezetimibe affects adipose tissue hormonal function. This tissue is more than just a passive repository for excess energy. It is a highly active metabolic and endocrine organ secreting a range of bioactive peptides with both local and distant action collectively called 'adipokines' or 'adipose tissue hormones' [11,12]. Because at least some adipokines are involved in regulating energy homeostasis, carbohydrate and lipid metabolism, vascular homeostasis and immune response, their abnormal secretion may contribute to the development of atherosclerosis and its complications [11,12]. Leptin, adiponectin, visfatin and tumor necrosis factor-α (TNF-α) were chosen for investigation because their increased (leptin, visfatin, TNF-α) or reduced (adiponectin) concentrations are strongly associated with an enhanced risk of atherosclerosis and its complications [13,14].

## Materials and methods

Patients (35–60 years old), recruited between February and December 2012, were eligible for enrollment if they had isolated hypercholesterolemia, defined as total plasma cholesterol above 200 mg/dL, LDL cholesterol more than 130 mg/dL and triglycerides below 150 mg/dL, and could not be treated with HMG-CoA reductase inhibitors because of either statin intolerance or having contraindications to this form of treatment. We excluded patients with any acute and chronic inflammatory processes, stage 2 or 3 hypertension (according to the 2003 European Society of Hypertension–European Society of Cardiology guidelines), unstable coronary artery disease, myocardial infarction or stroke within 6 months preceding the study, symptomatic congestive heart failure, diabetes, autoimmune disorders, thyroid diseases, chronic pancreatitis, impaired renal or hepatic function, nephrotic syndrome and with body mass index above 35 kg/m$^2$. No patient was treated with other hypolipidemic drug within 3 months before the study. All included patients ($n = 22$) were treated with ezetimibe (10 mg), which was administered once daily for 90 days without any changes in dosage throughout the study. Throughout the study the participants complied with lifestyle modifications. On the basis of the result of HOMA-IR index, the patients were divided into two subgroups, with 'normal' or 'disturbed' insulin sensitivity. Normal insulin sensitivity was arbitrarily defined as HOMA-IR less than 2.5. If this value was exceeded, the patient was diagnosed as insulin-resistant. Two control groups included respectively 20 age-, sex-, weight-, blood pressure-matched statin-intolerant subjects with isolated hypercholesterolemia following lifestyle modification, but not treated with any hypolipidemic agent, as well as 20 matched healthy subjects recruited among staff and relatives of the patients. The investigation of possible ezetimibe-induced side-effects was performed every two weeks. Compliance was assessed during each visit by tablet counts and was considered satisfactory when the number of tablets taken by a patient ranged from 90% to 110%. The Bioethical Committee of the Medical University of Silesia approved the study protocol. All enrolled patients provided their written informed consent for the investigation and the study was performed according to the Declaration of Helsinki.

Lipid profile, and plasma levels of glucose, insulin, CRP, total non-estrified free fatty acids (FFA), leptin, adiponectin, visfatin and tumor necrosis factor-α (TNF-α) were determined before and after 30 and 90 days of therapy. Blood samples were taken after an overnight fast in a quiet, temperature-controlled room (24–25 °C) between 8.00 and 9.00 a.m. (to avoid circadian fluctuations of the parameters studied). Patients were asked to refrain from smoking and from taking vigorous exercise prior to blood sampling. After obtaining fasting blood samples, all patients underwent a 75-g oral glucose tolerance test. The plasma samples were separated and stored at −70 °C until analysis, which was performed by a person blinded to subject identity and all clinical details. To minimize analytical errors, all assays were performed in duplicate. Routine methods were used to determine plasma concentrations of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, apolipoproteins A-I and B-100, glucose and insulin (bioMerieux France; Inctstar Corporation, Stillwater, MN; Beckman, Palo Alto, CA; Linco Research Inc, St Charles, MO). Fasting plasma glucose and insulin levels were used to calculate the homeostatic model assessment of insulin resistance ratio (HOMA-IR) [fasting serum glucose (mg/dL) × fasting insulin level (μU/mL)/405]. FFA were measured by an enzymatic assay using reagents from Alpha Laboratories (Eastleigh, Hants, UK). Plasma levels of CRP were measured using a high-sensitivity monoclonal antibody assay (hsCRP) (MP Biomedicals, Orangeburg, NY). Plasma levels of leptin, adiponectin, visfatin and TNF-α were measured with commercial enzyme-linked immunosorbent assay kits obtained from TECOmedical Group (Sissach, Switherland), Phoenix Pharmaceuticals (Burlingame, CA) and R&D Systems (McKinley Place N.E., Minneapolis, MN). The minimum detectable levels for the assessed parameters were: 0.1 mg/L, 7.8 pg/mL, 0.246 ng/mL, 6.1 pg/mL and 1.6 pg/mL, respectively for hsCRP, leptin, adiponectin, visfatin and TNF-α. The intra- and interassay coefficients of variation in our laboratory were as follows: hsCRP – 4.1% and 5.6%, leptin – 3.4% and 5.5%, adiponectin – 3.4% and 6.0%, visfatin – 5.5% and 7.3%, TNF-α – 4.4% and 8.7%.

Because of the skewed distributions, values for the HOMA-IR, hsCRP, FFA and cytokines were natural-log transformed to satisfy assumptions of normality and equal variance. Comparisons between the groups were performed using one-way ANOVA followed by Bonferroni's post hoc analysis. Student's paired *t*-test was applied to compare pre-, inter- and post-treatment data within the same group. For categorical variables $\chi^2$ test was used. Kendall's tau test was used to evaluate the relationships between variables. Statistical significance was assumed at $p$ less than 0.05.

## Results

At baseline, all groups were comparable with respect to sex distribution, age, body weight, and medical background (Table 1). Compared to healthy subjects, both groups of isolated hypercholesterolemic patients had higher plasma levels of hsCRP, FFA, leptin, visfatin and TNF-α, as well as lower plasma levels of adiponectin.

One ezetimibe-treated patient was withdrawn from the study because of an increase in alanine and aspartate aminotransferase activities. Two non-pharmacologically treated patients with hipercholesterolemia did not comply with the study protocol and resigned from further participation in the study. No serious adverse events were observed throughout the study in the remaining patients who completed the study protocol.

In healthy subjects and non-treated patients with isolated hypercholesterolemia, lipid profile, glucose homeostasis markers and plasma levels of hsCRP, FFA and of all investigated adipokines remained at the similar level throughout the study.

Ezetimibe treatment decreased plasma levels of total cholesterol by 22% ($p < 0.001$) and 23% ($p < 0.001$), LDL cholesterol by 26% ($p < 0.001$) and 27% ($p < 0.001$) and apolipoprotein B-100 by 18% ($p < 0.001$) and 20% ($p < 0.001$), respectively, after 30 days and at the end of the study (Table 2). Ninety, but not 30-day, ezetimibe decreased plasma levels of visfatin (by 26%, $p < 0.05$), as well as tended to reduce plasma levels of leptin (−22%, $p = 0.095$) and TNF-α (−21%, $p = 0.052$). Ezetimibe administration to hypercholesterolemic patients tended to reduce plasma levels of hsCRP by 18%