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The effect of short-term simvastatin treatment on plasma adipokine levels in patients with isolated hypercholesterolemia: A preliminary report

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ABSTRACT

Background: Apart from reducing plasma lipids, statins produce numerous non-lipid-related pleiotropic effects. The aim of this study was to investigate whether short-term simvastatin treatment affects plasma adipokine levels in patients with isolated hypercholesterolemia.
Methods: The study included 42 adult patients with untreated isolated hypercholesterolemia, complying throughout the study with lifestyle intervention, 23 of whom were treated with simvastatin (40 mg daily), as well as 18 healthy subjects with normal lipid profile. Plasma lipids, apolipoproteins, glucose metabolism markers, as well as plasma levels of C-reactive protein (CRP), free fatty acids (FFA), leptin, adiponectin, visfatin and tumor necrosis factor-α (TNF-α) were determined at baseline and after 30 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, TNF-α, FFA and CRP, as well as lower plasma levels of adiponectin. Apart from decreasing plasma total cholesterol, LDL cholesterol and apolipoprotein B-100 levels, simvastatin reduced plasma levels of FFA, leptin and TNF-α, as well as increased plasma levels of adiponectin, which was accompanied by a reduction in plasma CRP. There were no differences in simvastatin action on plasma adipokines and CRP between insulin-resistant and insulin-sensitive subjects.
Conclusions: Our results indicate that the presence of isolated hypercholesterolemia is associated with abnormal hormonal function of the adipose tissue. These changes are partially reversed by short-term simvastatin treatment, and this action may contribute to the clinical effectiveness of statins in the therapy of atherosclerosis-related disorders.

Introduction

Recent large clinical trials have demonstrated that 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, markedly reduce morbidity and mortality when used in the primary and secondary prevention of cardiovascular diseases. Apart from lowering lipids, statins 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 [1,2]. These so-called pleiotropic effects may partially explain why the benefits of statin therapy are observed soon after the start of therapy, though no or only minimal atherosclerotic plaque regression can be visualized by coronarography [3,4].

For decades, adipose tissue had been regarded only as a storage depot for body energy, mechanical defense against injuries, and a thermoregulator. The discovery of adipose tissue products, known as 'adipokines' or 'adipose tissue hormones', markedly broadened our knowledge on the contribution of adipose tissue to whole-body homeostasis and this tissue is now recognized to play a central role in the pathophysiology of insulin resistance and metabolic syndrome [5]. Because at least some adipokines are involved in

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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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 [6,7].

Unfortunately, studies assessing the effect of statin treatment on plasma adipokines provided contrasting results. Some authors [8–11] observed that administration of HMG-CoA reductase inhibitors is associated with beneficial changes in adipose tissue function, while others [12–15] did not observe any effect of statins on plasma adipokines. These discrepancies may be attributed to differences in inclusion criteria, type of statin and its dosage, the duration of treatment, diurnal and/or seasonal variations in adipokine production as well as methodological differences.

In this short study, we assessed the effect of a 30-day treatment with simvastatin on the plasma adipokine levels and low-grade inflammation in patients with elevated LDL cholesterol levels. We chose 30 days as a treatment period because it was the shortest time after which in our pilot studies simvastatin increased plasma adiponectin levels (no effect was observed after 7 and 15 days) (Krysiak et al., unpublished observations). Leptin, adiponectin, visfatin and tumor necrosis factor-α (TNF-α) were selected for investigation because their abnormal values are strongly associated with an enhanced risk of atherosclerosis and its complications [16,17].

Materials and methods

The study complied with the principles of the Declaration of Helsinki and its protocol was approved by the Bioethical Committee of the Medical University of Silesia. All included patients gave their written informed consent to participate in the study. The study population included 23 simvastatin-treated subjects (35–60 years old) with isolated hypercholesterolemia, defined as total plasma cholesterol above 200 mg/dL, LDL cholesterol above 130 mg/dL and triglycerides below 150 mg/dL. All patients had been complying with lifestyle intervention but had not received any hypolipidemic agent for at least 3 months before the beginning of the study. 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. Simvastatin was administered at the daily dose of 40 mg once daily at bedtime for 30 days and no changes in medication dosage were allowed throughout the study. Throughout the entire study period, all participants continued to comply 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 defined as HOMA-IR less than 2.5. If HOMA-IR was above this value, the patient was diagnosed as insulin-resistant. Simvastatin-treated patients were compared with 19 age-, sex- and weight-matched hypercholesterolemic patients following lifestyle modification but not treated with any hypolipidemic agent, as well as with a group of 18 healthy subjects with normal plasma lipids. Compliance was monitored during each visit by tablet count and was regarded as satisfactory if the number of tablets taken by a patient ranged from 90% to 110%.

To avoid diurnal variations in the parameters studied, all blood samples were taken between 8.00 and 9.00 a.m. after a 12-h overnight fast in a quiet, temperature-controlled room (24–25 °C). The samples were immediately coded so that the person performing laboratory assay was blind to subject identity and study sequence. Moreover, blood samples were also taken 2 h after a 75 g oral glucose load. All measurements were performed in duplicate (to minimize analytical errors) according to manufacturers' recommendations. Plasma lipids (total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides), apolipoproteins A-I and B-100, fasting and 2-h post-challenge plasma glucose concentrations, plasma insulin, free fatty acids (FFA) and hsCRP, as well as all investigated cytokines were determined at entry and after 30 days of therapy. Plasma lipids, glucose, and insulin were assayed by routine laboratory techniques (bioMerieux France; Beckman, Palo Alto, CA; Linco Research, St. Charles, MO; Bayer Ames Technicon, Tarrytown, NY). The homeostasis model of insulin resistance (HOMA-IR) index was calculated as [fasting plasma glucose (mg/L) × fasting plasma insulin (μU/mL)/405]. Total non-esterified 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, Switzerland), Phoenix Pharmaceuticals (Burlingame, CA) and R&D Systems (McKinley Place N.E. Minneapolis, Minnesota). 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%.

The distribution of the variables was analyzed using the Shapiro–Wilk test. In the case of variables with non-normal distribution (HOMA-IR, hsCRP, adipokines), log transformation was used to fit a normal distribution curve. Between-group comparisons were performed using one-way ANOVA followed by the *post hoc* Bonferroni test (when three groups were compared) or using independent samples *t*-test (when two groups were compared). The differences between baseline and post-treatment values within the same treatment group were compared with the Student's paired *t*-test. Correlations between the study parameters were calculated using Kendall's tau test. *P*-value less than 0.05 was considered significant. Analyses were conducted using GraphPad Prism 2.01 software and Statistica 6.1.

Results

There were no significant differences in the age, weight and sex between all investigated groups. Expectedly, patients with isolated hypercholesterolemia exhibited higher baseline plasma levels of total cholesterol, LDL-cholesterol and apolipoprotein B-100 (Table 1). Compared to the healthy subjects, patients with hypercholesterolemia were characterized by higher plasma levels of hsCRP, FFA, leptin, visfatin and TNF-α, and lower plasma levels of adiponectin.

One subject treated with simvastatin was withdrawn from the study because of myalgia. Two individuals with hypercholesterolemia not treated pharmacologically dropped out due to non-compliance with the study protocol. Neither significant adverse effects nor any complications were reported throughout the entire study period in the remaining participants. Thirty days of simvastatin treatment and lifestyle modification had no effect on body mass index and waist circumference (data not shown).

In the hypercholesterolemic patients not treated pharmacologically and in the healthy subjects, plasma lipids/lipoproteins, glucose homeostasis markers, as well as plasma levels of hsCRP, FFA, leptin, adiponectin, visfatin and TNF-α remained unaltered throughout the study (Tables 2 and 3). Baseline plasma levels of