Comparison of effects of bezafibrate and fenofibrate on circulating proprotein convertase subtilisin/kexin type 9 and adipocytokine levels in dyslipidemic subjects with impaired glucose tolerance or type 2 diabetes mellitus: Results from a crossover study

Tohru Noguchi[1], Junji Kobayashi[1*], Kunimasa Yagi[2], Atsushi Nohara[1], Naoto Yamaaki[2], Masako Sugihara[2], Naoko Ito[2], Rie Oka[3], Masa-aki Kawashiri[2], Hayato Tada[2], Mutsuko Takata[2], Akihiro Inazu[4], Masakazu Yamagishi[2], Hiroshi Mabuchi[1]

[1]Department of Lipidology, Graduate School of Medical Science, Kanazawa University, Japan
[2]Division of Cardiovascular Medicine, Graduate School of Medical Science, Kanazawa University, Japan
[3]Department of Internal Medicine, Hokuriku Chuo Hospital, Japan
[4]Laboratory Science, Graduate School of Medical Science, Kanazawa University, Japan

Corresponding author: Junji Kobayashi

Department of Lipidology, Graduate School of Medical Science, Kanazawa University

Takara-machi 13-1, Kanazawa, 920-8640, Japan

Tel: +81-76-265-2268

Fax: +81-76-234-4271

E-mail: junji@med.kanazawa-u.ac.jp

Abstract

Background: Bezafibrate and fenofibrate show different binding properties against peroxisome proliferator-activated receptor subtypes, which could cause different clinical effects on circulating proprotein convertase subtilisin/kexin type 9 (PCSK9) levels and on various metabolic markers. **Methods:** An open, randomized, four-phased crossover study using 400mg of bezafibrate or 200mg of fenofibrate was performed. Study subjects were 14 dyslipidemia with impaired glucose tolerance or type 2 diabetes mellitus (61 ± 16 years, body mass index (BMI) 26 ± 3 kg/m^2, total cholesterol (TC) 219 ± 53 mg/dL, triglyceride (TG) 183 ± 83 mg/dL, high-density lipoprotein-cholesterol (HDL-C) 46 ± 8 mg/dL, fasting plasma glucose 133 ± 31 mg/dL and HbA1c $6.2 \pm 0.8\%$). Subjects were given either bezafibrate or fenofibrate for 8 weeks, discontinued for 4 weeks and then switched to the other fibrate for 8 weeks. Circulating PCSK9 levels and other metabolic parameters, including adiponectin, leptin and urine 8-hydroxy-2'-deoxyguanosine (8-OHdG) were measured at 0, 8, 12 and 20 weeks. **Results:** Plasma PCSK9 concentrations were significantly increased (+39.7% for bezafibrate and +66.8% for fenofibrate, $p < 0.001$) in all patients except for one subject when treated with bezafibrate. Both bezafibrate and fenofibrate caused reductions in TG (-38.3%, $p < 0.001$ $vs.$ -32.9%, $p < 0.01$) and increases in HDL-C (+18.0%, $p < 0.001$ $vs.$ +11.7%, $p < 0.001$). Fenofibrate significantly reduced serum cholesterol levels (TC, -11.2%, $p < 0.01$; non-HDL-C, -17.3%, $p < 0.01$; apolipoprotein B, -15.1%, $p < 0.01$), whereas bezafibrate significantly improved glucose tolerance (insulin, -17.0%, $p < 0.05$) and metabolic markers (γ-GTP, -38.9% $p < 0.01$; adiponectin, +15.4%, $p < 0.05$; urine 8-OHdG/Cre, -9.5%, $p < 0.05$). **Conclusion:** Both bezafibrate and fenofibrate increased plasma PCSK9 concentrations. The addition of a PCSK9 inhibitor to each fibrate therapy may achieve beneficial cholesterol lowering along with desirable effects of respective fibrates.

Introduction

Proprotein convertase subtilisin kexin type 9 (PCSK9) is a member of the subtilisin-like serine convertase superfamily made and secreted by the liver into the plasma. Secreted PCSK9 regulates plasma LDL-cholesterol (LDL-C) levels by directing cell-surface LDL receptors (LDLR) to the lysosomes for degradation, resulting in reduced clearance and accumulation of LDL-C in the circulation [1-3]. Statins have been known to increase the nuclear translocation of sterol-regulatory element binding protein-2 (SREBP-2), which activates not only the LDLR but also PCSK9 gene expression [4-6]. Subsequently, statins increases serum PCSK9 levels [5-8] and this increment may attenuate the LDL-C lowering effect of statins. It may in a part explain the rule of 6% for statins, which indicates that each doubling of the statin dose results in only about a 6% further decrease in LDL-C.

Unlike statin treatment, the association of fibrate treatrment with PCSK9 is less clear. Recently, it was shown that fenofibrate suppressed PCSK9 expression at the promoter level and reduced statin-induced PCSK9 secretion in vitro [9], whereas, fenofibrate did not suppress and rather increased circulating PCSK9 level in several in vivo studies [8, 10, 11]. Bezafibrate is another widely used fibrate, and both of which have substantial triglyceride (TG)-reducing-effect and high-density lipoprotein cholesterol (HDL-C)-raising effect. Bezafibrate is a pan-agonist for peroxisome proliferator-activated receptor (PPAR)-α, β and δ and fenofibrate is a more selective ligand for PPAR-α [12], and thus the effects of these drugs varies in several aspects. To the best of our knowledge, there is no previous study on the effect of bezafibrate treatment on PCSK9.

In this background, we directly compared the effect of fenofibrate and bezafibrate treatment on plasma PCSK9 and other metabolic parameters related to insulin sensitivity in crossover design.

2. Materials and Methods

2.1. Study subjects

To assess the effects of bezafibrate and fenofibrate on plasma PCSK9, glucose tolerance and lipid metabolism, we enrolled 14 dyslipidemic subjects with impaired glucose tolerance or type 2 diabetes mellitus (T2DM); 11 men and 3 women, age 60.5 ± 15.6 years, body mass index (BMI) 26.0 ± 3.1 kg/m^2, total cholesterol (TC) 219.0 ± 52.7 mg/dL, TG 183.0 ± 82.9 mg/dL, HDL-C 45.9 ± 8.4 mg/dL, fasting plasma glucose 133.0 ± 30.8 mg/dL and HbA1c $6.22 \pm 0.85\%$. Among them, 5 were on anti-diabetic drug and 7 were on anti-hypertensive drug, and the dosages of these treatments were not changed during the study period. None of the subjects were on insulin treatment or anti-lipid drugs other than fibrates.

Exclusion included: age > 75 years, BMI > 30 kg/m^2, HbA1c > 7.5%, serum TG level > 400mg/dL, abnormal liver or muscle enzymes, creatinemia, habitual alcohol intake > 3 standard drink/day or endocrinological disorder.

2.2. Study protocol

This study was conducted as open randomized crossover design to compare the efficacy and the safety of bezafibrate and fenofibrate. All the participants were outpatients and were divided into two groups by envelope-method (Table 1). The group 1 (6 male, average age of 63.0 years) was started with 400 mg/day of bezafibrate, while the group 2 (5 male and 3 female, average age of 58.6 years) with 200mg/day of fenofibrate. Any lipid lowering agents had been discontinued at least for 4 weeks before the study. The first phase was continued for 8 weeks, then bezafibrate or fenofibrate were discontinued for 4 weeks, and then subsequently patients were switched to the other fibrate treatment and continued it for another 8 weeks.

A statement of institutional approval of the study in accordance with the Declaration of Helsinki

was provided, and written informed consent was obtained from all of the participants in this study.

2.3. Measurement of the laboratory data

Blood samples were obtained after an overnight fasting, and centrifuged at 4 °C. Serum TC and TG were determined by enzymatic method, and HDL-C levels were measured by a polyamine-polymer/detergent method (Daiichi Pure Chemical, Tokyo, Japan) as described elsewhere [13]. LDL-C levels were calculated using the Friedewald formula. Apolipoprotein A1, A2, B, C2, C3 and E were determined as described previously [14]. Plasma PCSK9 concentrations were determined using a commercially available quantitative sandwich enzyme-linked immunosorbent assay (ELISA) kit targeting human PCSK9 following the manufacturer instructions (Circulex CY-8079, CycLex Co, Nagano, Japan). The plasma levels of adiponectin and leptin were determined by the previously reported methods [15, 16]. The levels of Urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) were determined using an ELISA kit (Stressgen, British Columbia, Canada) in accordance with the manufacturer's instructions. Other laboratory values, including fasting plasma glucose, HbA1c, glycoalbumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (γ-GTP) were obtained using commercially available kits with an autoanalyzer. Serum insulin levels were determined by enzyme immunoassay. Uric Acid was determined using the uricase method. Homeostasis model assessment-Insulin Resistance (HOMA-IR) was calculated using the formula: HOMA-IR = (fasting insulin in mU/l × fasting plasma glucose in mg/dl)/405.

2.4. Statistics

All values in the text and tables are expressed as mean ± SD unless otherwise stated. Effects of each drug therapy on each variable were analyzed by means of paired *t*-test. Treatment effects were

compared between two fibrates using a mixed model ANOVA. Linear correlations were analyzed using Pearson's correlation coefficient analysis. All statistical analyses were performed with PASW statistics 17.0.3 (SPSS, Chicago, Illinois). A p-value of less than 0.05 was considered to indicate statistical significance.

3. Results

3.1. Characteristics of study subjects at baseline were similar in both groups

In either group 1 or 2, parameters related to lipid, glycemic control and other markers did not significantly differ before starting either fibrate. For further analysis, the data from both groups were combined and analyzed in detail. Pre- and post-treatment values of each parameter were shown in Table 2. There were no variables that would indicate carry-over and time-dependent effects.

3.2. Bezafbrate decreased γ-GTP, whereas fenofibrate showed better effect on uric acid than bezafibrate

There was no significant change in BMI and AST between values before and after treatment. There was a slight but not significant reduction in ALT during either treatment. γ-GTP decreased with bezafibrate (p < 0.01), but not with fenofibrate. Uric acid decreased with fenofibrate (p < 0.001), whereas slightly increased with bezafibrate (p < 0.05), and the effects of two fibrates on uric acid were significantly different from each other (p < 0.001).

3.3. Fenofibrate had better effects on cholesterol metabolism than bezafibrate, though both fibrates increased plasma PCSK9 levels

Plasma PCSK9 levels were increased in 13 of 14 subjects with bezafibrate (+39.7%, p < 0.001, Figure 1A) and in all subjects with fenofibrate (+66.8%, p < 0.001, Figure 1B).

Both bezafibrate and fenofibrate treatments were associated with reductions in serum TG (bezafibrate, $p < 0.001$; fenofibrate $p < 0.01$), and increases in HDL-C ($p < 0.001$ for either of treatment). There were significant reductions in TC ($p < 0.01$), apolipoprotein B ($p < 0.01$) and non-HDL-C ($p < 0.01$), and slight decrease in LDL-C during fenofibrate treatment, whereas bezafibrate did not produce reductions in these parameters. As a result, cholesterol lowering effect of fenofibrate were significantly stronger than that of bezafibrate ($p < 0.05$ for TC, LDL-C and apolipoprotein B).

Serum levels of apolipoprotein A1 and A2 were significantly increased ($p < 0.001$) and those of apolipoprotein C3 were significantly decreased ($p < 0.01$) during both of these treatments. Apolipoprotein E level was slightly but significantly decreased during bezafibrate treatment ($p < 0.05$). There were no significant changes in apolipoprotein C2 levels between values before and after fenofibrate or bezafibrate treatments.

3.4. Bezafibrate had better effects on glucose tolerance than fenofibrate

Changes in plasma glucose and glycoalbumin were not significant during either treatment. Fenofibrate was associated with a slight but significant increase in HbA1c ($p < 0.05$) whereas bezafibrate was not. Insulin level and HOMA-IR significantly decreased with bezafibrate ($p < 0.05$) but not with fenofibrate.

3.5. Bezafibrate improved serum adiponectin level and oxidative stress marker

Bezafibrate but not fenofibrate was associated with a significant increase in serum adiponectin ($p < 0.05$) and with a significant decrease in urine 8-OHdG/Cre ($p < 0.05$). Serum leptin did not significantly alter in either treatment.

3.6. The associations of plasma PCSK9 with cholesterol and several metabolic markers

As shown in Table 3, circulating PCSK9 levels were significantly correlated with TC (r = 0.477, *p* = 0.01), LDL-C (r = 0.501, *p* < 0.01), non-HDL-C (r = 0.453, *p* < 0.05), BMI (r = 0.377, *p* < 0.05), γGTP (r = 0.376, *p* < 0.05), plasma glucose (r = -0.426, *p* < 0.05), HbA1c (r = -0.468, *p* < 0.05), glycoalbumin (r = -0.563, *p* < 0.01), insulin (r = 0.507, *p* < 0.01), HOMA-IR (r = 0.396, *p* < 0.05) and leptin (r = 0.416, *p* < 0.05) at baseline levels. When analyzed using absolute or percent change value for each variable during the treatment, no significant associations were observed in the present study.

4. **Discussion**

The main finding of the present study is that all subjects with fenofibrate treatment and all but one subjects with bezafibrate treatment showed increases in their circulating PCSK9 levels. The degree of increase in PCSK9 tended to be higher in fenofibrate treatment than in bezafibrate treatment (+66.8% *vs*. +39.7%, *p* = 0.077). This is the first study investigating the differences between bezafibrate and fenofibrate in their effects on circulating PCSK9 level and other metabolic parameters related to insulin resistance and oxidative stress in Japanese hyperlipidemic subjects.

As observed in non-diabetic patients [17-19], we found that plasma PCSK9 levels positively correlated with TC, LDL-C, non-HDL-C, BMI, γGTP, insulin and HOMA-IR at baseline levels in subjects with impaired glucose tolerance or T2DM. On the other hand, fasting plasma glucose was negatively correlated with plasma PCSK9 levels in our study on impaired glucose tolerance or type 2 diabetes mellitus, which does not appear to be consistent with these reports on non-diabetic patients. Very recently, however, it was shown that PCSK9 knockout mice had higher glucose and lower insulin levels than wild-type mice [20], suggesting some association of PCSK9 with increasing insulin leading to lowering plasma glucose. This may in part explain the negative correlation of

8

PCSK9 with plasma glucose and HbA1c in our study.

Bezafibrate and fenofibrate have some evidence of preventing coronary artery disease in Bezafibrate Infarction Prevention (BIP) [21, 22] and Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study [23], respectively. Our present data indicate that fenofibrate treatment (200 mg per day for 8 weeks) produced more favorable effects on cholesterol metabolism than did bezafibrate treatment (400 mg per day for 8 weeks) with regard to lower TC, non-HDL-C, LDL-C and apolipoprotein B levels. Circulating PCSK9 protein levels were significantly increased by either of these two fibrates,

Previous reports showed conflicting findings on the effect of fenofibrate on PCSK9. Kourimate et al. demonstrated that PPAR-α activation resulted in the repression of PCSK9 promoter activity, and in the up-regulation of PC5/6A and furin, which degrade PCSK9 [9]. To our knowledge, two clinical studies suggest that fenofibrate treatment was associated with decrease in PCSK9 levels [24, 25]. Lambert et al. [24] reported that fenofibrate 200 mg/day was associated with decrease (-8.5%; $p = 0.041$) in PCSK9 level in diabetic patients and Chan et al. [25] showed fenofibrate 145 mg/day was associated with decrease (-13%; $p < 0.05$) in statin treated T2DM subjects. In contrast to these reports, Mayne et al. and Troutt et al. represented an increase in circulating PCSK9 level after fenofibrate treatment with 200 mg/day in dyslipidemic patients (+17%; $p = 0.031$ and +25%; $p < 0.01$, respectively) [8, 10], although the precise mechanism contributing to these increases remains to be clarified. Furthermore, Costet et al. reported that 6 weeks of treatment with either 10 mg/day atorvastatin or 160 mg/day fenofibrate increased PCSK9 levels (+14%; $p = 0.01$ and +26%; $p < 0.01$, respectively) in T2DM subjects, with no additive effect after 6 weeks of combined therapy [11]. In our study, the effect of fenofibrate treatment with 200 mg/day on plasma PCSK9 level was more prominent (+66.8%) than these reports and was even higher than previously reported value of high dose (80 mg) atorvastatin treatment (+47%) [26], although study population and duration of the

treatment were different.

In patients with T2DM, insulin resistance inhibits catabolism of remnant lipoproteins by reducing lipoprotein lipase (LPL) activity, contributing to the development of hypertriglyceridemia and low HDL-C [27-29]. The present data indicate that bezafibrate and fenofibrate had equivalent efficacy on TG and HDL-C improvement in subjects with hyperglycemia. As a PPAR-α agonists, these fibrates improve triglyceride and HDL-C concentrations by enhancing β-oxidation of fatty acids and LPL activity, increasing production of the components of HDL (apolipoproteins A1 and A2), and reducing production of the inhibitor of LPL activity (apolipoprotein C3) [30-34], while enhancing cholesterol efflux from the liver [35]. Several reports [8, 10] suggest that these effects of PPAR-α on cholesterol and lipoprotein metabolism may lead indirectly to decrease hepatic intracellular cholesterol levels, and thus result in a secondary increase in PCSK9 expression and secretion. Costet et al. reported that one-day administration of atorvastatin increased PCSK9 levels whereas that of fenofibrate did not [11], which might support the indirect slower effect of PPAR-α agonist on PCSK9 expression.

PPAR-γ is another target of bezafibrate and is expected to have effects on glucose metabolism and insulin resistance [36]. Several previous studies have indicated that bezafibrate treatment could be associated with improvement of glycemic control in diabetic subjects [37, 38] or with delay of the onset of T2DM [39, 40]. As for fenofibrate treatment, on the other hand, there are conflicting reports on its effect on glycemic control [41, 42]. In our previous study [43], fasting plasma glucose level was unchanged after 8 weeks of treatment with fenofibrate, which is consistent with the present study. Of note, the very recent retrospective cohort study using data from routine medical practice in the United Kingdom [44] has shown that compared to fenofibrate, bezafibrate was associated with a particularly low hazard for the occurrence of T2DM (HR 0.41, 95% CI 0.29, 0.58). Indeed, our present findings suggest that bezafibrate is more desirable than fenofibrate concerning serum insulin level and HbA1c.

The mechanism by which the degree of PCSK9 increases was more modest in bezafibrate than in fenofibrate is not clarified yet. Costet et al. reported that insulin increased hepatic PCSK9 mRNA expression in rodent primary hepatocytes and in vivo during a hyperinsulinemic-euglycemic clamp in mice [45]. This finding combined with our present result that insulin level positively correlated with plasma PCSK9 level at baseline and that insulin level was decreased with bezafibrate but not with fenofibrate may in part explain the more modest increase in PCSK9 levels produced by the former than the latter.

In addition to the effect on glycemic control, these fibrates may have beneficial effects on adipocytokines, such as leptin, which positively correlated with PCSK9 in the present study, and adiponectin [41, 46-49]. Damci et al. showed a reduction in leptin levels with fenofibrate treatment (250 mg/day for 3 months) [41], whereas no significant change in leptin levels was observed in either fibrate treatment in the present study with a shorter duration. For the effect of fibrate treatment on serum adiponectin, the present finding that fenofibrate did not increase serum adiponectin is consistent with our previous study [43], although some studies have shown associations of fenofibrate treatment with adiponectin increase in humans [46, 47]. On the other hand, bezafibrate treatment was associated with an increase in serum adiponectin in the present study. Indeed, there are several previous studies linking bezafibrate to an increase in serum adiponectin in patients enrolled in the BIP study [48] and in spontaneous T2DM model (Otsuka Long-Evans Tokushima Fatty; OLETF) rats [49]. Hiuge et al. also reported that bezafibrate and fenofibrate significantly increased adiponectin levels in mice and 3T3-L1 adipocytes [48].

For the association between fibrate and liver function, there is a report linking bezafibrate treatment to an improvement of γ-GTP in subjects with chronic liver disease [50], which is compatible with the present finding although subjects were not chronic liver disease in our study. Consistent with the report by Cariou et al. [18], γ-GTP positively correlated with PCSK9 at baseline in our study, and

11

thus the significant decrease in γ-GTP only with bezafibrate might be related to the milder increase in circulating PCSK9 levels with bezafibrate than fenofibrate. A significant reduction in uric acid levels by fenofibrate in the present study is consistent with previous report [51], whereas there is no previous report on the effect of bezafibrate treatment on uric acid.

Angiopathy of T2DM has a complex etiology that may involve the effects of hyperlipidemia and oxidative stress on endothelial function. Similar to certain types of lipid lowering agents such as probucol and atorvastatin [52], we observed beneficial effect of bezafibrate to produce significant decreases in urine 8-OHdG/Cre, which has been recognized as an important oxidative stress marker.

One of the limitations of our study is the small sample size. However, the study was accomplished in the cross over design, making it possible to investigate the effect of bezafibrate and fenofibrate treatments in the same subjects and thereby enabling to exclude the possibility of study subject bias. Moreover, the results were clear and convincing especially in PCSK9 changes during fibrate treatment.

In conclusion, both bezafibrate and fenofibrate cause considerable increase in plasma PCSK9 in hyperlipidemic subjects with the former in more modest manner. We suggest that in the treatment of hyperlipidemic subjects, the addition of a PCSK9 inhibitor to each fibrate therapy may achieve beneficial cholesterol lowering along with desirable effects of respective fibrates.

Acknowledgement

Disclosure

The contents of this paper have not been published or communicated elsewhere. The authors have no relationships with companies regarding a financial interest in the information contained in this paper.

References

[1]   Zhang DW, Lagace TA, Garuti R, et al. Binding of proprotein convertase subtilisin/kexin type 9 to epidermal growth factor-like repeat A of low density lipoprotein receptor decreases receptor recycling and increases degradation. J Biol Chem 2007; 282:18602-18612.

[2]   Qian YW, Schmidt RJ, Zhang Y, et al. Secreted PCSK9 downregulates low density lipoprotein receptor through receptor-mediated endocytosis. J Lipid Res 2007; 48:1488-1498.

[3]   Lagace TA, Curtis DE, Garuti R, et al. Secreted PCSK9 decreases the number of LDL receptors in hepatocytes and in livers of parabiotic mice. J Clin Invest 2006; 116:2995-3005.

[4]   Rashid S, Curtis DE, Garuti R, et al. Decreased plasma cholesterol and hypersensitivity to statins in mice lacking Pcsk9. Proc Natl Acad Sci U S A 2005; 102:5374-5379.

[5]   Dubuc G, Chamberland A, Wassef H, et al. Statins upregulate PCSK9, the gene encoding the proprotein convertase neural apoptosis-regulated convertase-1 implicated in familial hypercholesterolemia. Arterioscler Thromb Vasc Biol 2004; 24:1454-1459.

[6]   Dong B, Wu M, Li H, et al. Strong induction of PCSK9 gene expression through HNF1alpha and SREBP2: Mechanism for the resistance to LDL-cholesterol lowering effect of statins in dyslipidemic hamsters. J Lipid Res 2010; 51:1486-1495.

[7]   Careskey HE, Davis RA, Alborn WE, Troutt JS, Cao G, Konrad RJ. Atorvastatin increases human serum levels of proprotein convertase subtilisin/kexin type 9. J Lipid Res 2008; 49:394-398.

[8]   Mayne J, Dewpura T, Raymond A, et al. Plasma PCSK9 levels are significantly modified by statins and fibrates in humans. Lipids Health Dis 2008; 7:22.

[9]   Kourimate S, Le May C, Langhi C, et al. Dual mechanisms for the fibrate-mediated repression of proprotein convertase subtilisin/kexin type 9. J Biol Chem 2008; 283:9666-9673.

[10]   Troutt JS, Alborn WE, Cao G, Konrad RJ. Fenofibrate treatment increases human serum proprotein convertase subtilisin kexin type 9 levels. J Lipid Res 2010; 51:345-351.

[11]   Costet P, Hoffmann MM, Cariou B, Guyomarc'h Delasalle B, Konrad T, Winkler K. Plasma PCSK9 is increased by fenofibrate and atorvastatin in a non-additive fashion in diabetic patients. Atherosclerosis 2010; 212:246-251.

[12]   Willson TM, Brown PJ, Sternbach DD, Henke BR. The PPARs: from orphan receptors to drug discovery. J Med Chem 2000; 43:527-550.

[13]   Okazaki M, Sasamoto K, Muramatsu T, Hosaki S. Evaluation of precipitation and direct methods for HDL-cholesterol assay by HPLC. Clin Chem 1997; 43:1885-1890.

[14]   Labeur C, Shepherd J, Rosseneu M. Immunological assays of apolipoproteins in plasma: methods and instrumentation. Clin Chem 1990; 36:591-597.

[15]   Arita Y, Kihara S, Ouchi N, et al. Paradoxical decrease of an adipose-specific protein,

adiponectin, in obesity. Biochem Biophys Res Commun 1999; 257:79-83.

[16]   Hosoda K, Masuzaki H, Ogawa Y, et al. Development of radioimmunoassay for human leptin. Biochem Biophys Res Commun 1996; 221:234-239.

[17]   Dubuc G, Tremblay M, Pare G, et al. A new method for measurement of total plasma PCSK9: clinical applications. J Lipid Res 2010; 51:140-149.

[18]   Cariou B, Le Bras M, Langhi C, et al. Association between plasma PCSK9 and gamma-glutamyl transferase levels in diabetic patients. Atherosclerosis 2010; 211:700-702.

[19]   Lakoski SG, Lagace TA, Cohen JC, Horton JD, Hobbs HH. Genetic and metabolic determinants of plasma PCSK9 levels. J Clin Endocrinol Metab 2009; 94:2537-2543.

[20]   Mbikay M, Sirois F, Mayne J, et al. PCSK9-deficient mice exhibit impaired glucose tolerance and pancreatic islet abnormalities. FEBS Lett 2010; 584:701-706.

[21]   Tenenbaum A, Motro M, Fisman EZ, Tanne D, Boyko V, Behar S. Bezafibrate for the secondary prevention of myocardial infarction in patients with metabolic syndrome. Arch Intern Med 2005; 165:1154-1160.

[22]   Goldenberg I, Boyko V, Tennenbaum A, Tanne D, Behar S, Guetta V. Long-term benefit of high-density lipoprotein cholesterol-raising therapy with bezafibrate: 16-year mortality follow-up of the bezafibrate infarction prevention trial. Arch Intern Med 2009; 169:508-514.

[23]   Scott R, O'Brien R, Fulcher G, et al. Effects of fenofibrate treatment on cardiovascular disease risk in 9,795 individuals with type 2 diabetes and various components of the metabolic syndrome: the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study. Diabetes Care 2009; 32:493-498.

[24]   Lambert G, Ancellin N, Charlton F, et al. Plasma PCSK9 concentrations correlate with LDL and total cholesterol in diabetic patients and are decreased by fenofibrate treatment. Clin Chem 2008; 54:1038-1045.

[25]   Chan DC, Hamilton SJ, Rye KA, et al. Fenofibrate concomitantly decreases serum proprotein convertase subtilisin/kexin type 9 and very-low-density lipoprotein particle concentrations in statin-treated type 2 diabetic patients. Diabetes Obes Metab 2010; 12:752-756.

[26]   Welder G, Zineh I, Pacanowski MA, Troutt JS, Cao G, Konrad RJ. High-dose atorvastatin causes a rapid sustained increase in human serum PCSK9 and disrupts its correlation with LDL cholesterol. J Lipid Res 2010; 51:2714-2721.

[27]   Castro Cabezas M, de Bruin TW, de Valk HW, Shoulders CC, Jansen H, Willem Erkelens D. Impaired fatty acid metabolism in familial combined hyperlipidemia. A mechanism associating hepatic apolipoprotein B overproduction and insulin resistance. J Clin Invest 1993; 92:160-168.

[28]   De Man FH, Cabezas MC, Van Barlingen HH, Erkelens DW, de Bruin TW. Triglyceride-rich lipoproteins in non-insulin-dependent diabetes mellitus: post-prandial metabolism

and relation to premature atherosclerosis. Eur J Clin Invest 1996; 26:89-108.

[29]   Ginsberg HN. Insulin resistance and cardiovascular disease. J Clin Invest 2000; 106:453-458.

[30]   Rashid S, Watanabe T, Sakaue T, Lewis GF. Mechanisms of HDL lowering in insulin resistant, hypertriglyceridemic states: the combined effect of HDL triglyceride enrichment and elevated hepatic lipase activity. Clin Biochem 2003; 36:421-429.

[31]   Nagasawa T, Inada Y, Nakano S, et al. Effects of bezafibrate, PPAR pan-agonist, and GW501516, PPARdelta agonist, on development of steatohepatitis in mice fed a methionine- and choline-deficient diet. Eur J Pharmacol 2006; 536:182-191.

[32]   Goa KL, Barradell LB, Plosker GL. Bezafibrate. An update of its pharmacology and use in the management of dyslipidaemia. Drugs 1996; 52:725-753.

[33]   Tenenbaum A, Motro M, Fisman EZ. Dual and pan-peroxisome proliferator-activated receptors (PPAR) co-agonism: the bezafibrate lessons. Cardiovasc Diabetol 2005; 4:14.

[34]   Eisenberg S, Gavish D, Oschry Y, Fainaru M, Deckelbaum RJ. Abnormalities in very low, low and high density lipoproteins in hypertriglyceridemia. Reversal toward normal with bezafibrate treatment. J Clin Invest 1984; 74:470-482.

[35]   Keating GM, Croom KF. Fenofibrate: a review of its use in primary dyslipidaemia, the metabolic syndrome and type 2 diabetes mellitus. Drugs 2007; 67:121-153.

[36]   Lee CH, Olson P, Evans RM. Minireview: lipid metabolism, metabolic diseases, and peroxisome proliferator-activated receptors. Endocrinology 2003; 144:2201-2207.

[37]   Ogawa S, Takeuchi K, Sugimura K, et al. Bezafibrate reduces blood glucose in type 2 diabetes mellitus. Metabolism 2000; 49:331-334.

[38]   Tenenbaum A, Motro M, Fisman EZ, et al. Peroxisome proliferator-activated receptor ligand bezafibrate for prevention of type 2 diabetes mellitus in patients with coronary artery disease. Circulation 2004; 109:2197-2202.

[39]   Tenenbaum A, Motro M, Fisman EZ, et al. Effect of bezafibrate on incidence of type 2 diabetes mellitus in obese patients. Eur Heart J 2005; 26:2032-2038.

[40]   Tenenbaum H, Behar S, Boyko V, et al. Long-term effect of bezafibrate on pancreatic beta-cell function and insulin resistance in patients with diabetes. Atherosclerosis 2007; 194:265-271.

[41]   Damci T, Tatliagac S, Osar Z, Ilkova H. Fenofibrate treatment is associated with better glycemic control and lower serum leptin and insulin levels in type 2 diabetic patients with hypertriglyceridemia. Eur J Intern Med 2003; 14:357-360.

[42]   Tan CE, Chew LS, Tai ES, et al. Benefits of micronised Fenofibrate in type 2 diabetes mellitus subjects with good glycemic control. Atherosclerosis 2001; 154:469-474.

[43]   Asano A, Kobayashi J, Murase Y, et al. Effects of fenofibrate therapy on plasma

ubiquinol-10 and ubiquinone-10 levels in Japanese patients with hyperlipidemia and type 2 diabetes mellitus. Pharmacotherapy 2006; 26:447-451.

[44]   Flory JH, Ellenberg S, Szapary PO, Strom BL, Hennessy S. Antidiabetic action of bezafibrate in a large observational database. Diabetes Care 2009; 32:547-551.

[45]   Costet P, Cariou B, Lambert G, et al. Hepatic PCSK9 expression is regulated by nutritional status via insulin and sterol regulatory element-binding protein 1c. J Biol Chem 2006; 281:6211-6218.

[46]   Koh KK, Quon MJ, Han SH, et al. Additive beneficial effects of fenofibrate combined with atorvastatin in the treatment of combined hyperlipidemia. J Am Coll Cardiol 2005; 45:1649-1653.

[47]   Koh KK, Han SH, Quon MJ, Yeal Ahn J, Shin EK. Beneficial effects of fenofibrate to improve endothelial dysfunction and raise adiponectin levels in patients with primary hypertriglyceridemia. Diabetes Care 2005; 28:1419-1424.

[48]   Hiuge A, Tenenbaum A, Maeda N, et al. Effects of peroxisome proliferator-activated receptor ligands, bezafibrate and fenofibrate, on adiponectin level. Arterioscler Thromb Vasc Biol 2007; 27:635-641.

[49]   Mori Y, Oana F, Matsuzawa A, Akahane S, Tajima N. Short-term effect of bezafibrate on the expression of adiponectin mRNA in the adipose tissues: a study in spontaneously type 2 diabetic rats with visceral obesity. Endocrine 2004; 25:247-251.

[50]   Kita R, Takamatsu S, Kimura T, Kokuryu H, Osaki Y, Tomono N. Bezafibrate may attenuate biliary damage associated with chronic liver diseases accompanied by high serum biliary enzyme levels. J Gastroenterol 2006; 41:686-692.

[51]   Noguchi Y, Tatsuno I, Suyama K, et al. Effect of fenofibrate on uric acid metabolism in Japanese hyperlipidemic patients. J Atheroscler Thromb 2004; 11:335-340.

[52]   Endo K, Miyashita Y, Sasaki H, et al. Probucol and atorvastatin decrease urinary 8-hydroxy-2'-deoxyguanosine in patients with diabetes and hypercholesterolemia. J Atheroscler Thromb 2006; 13:68-75.

Figure legend

Figure 1. Changes in circulating PCSK9 concentrations in response to 8 weeks of treatment with (A) 400 mg of bezafibrate per day or (B) 200 mg of fenofibrate per day. Open circles represent means of 14 subjects and bars S.D. *** $p < 0.001$ *vs*. pre-treatment value.