Increased cholesterol absorption is associated with In-stent-restenosis after stent implantation for stable coronary artery disease

Sylvia Otto a,1, Dieter Lütjohann b,1, Anja Kerksiek b, Silvia Friedrichs b, Paul Christian Schulze a, Sven Möbius-Winkler a, Tudor C. Pörner 1, Oliver Weingärtner a, b

a Department of Internal Medicine I, Division of Cardiology, Pneumology, Angiology and Intensive Medical Care, University Hospital Jena, Friedrich-Schiller-University Jena, Germany
b Institute of Clinical Chemistry and Clinical Pharmacology, Medical Faculty, University of Bonn, Bonn, Germany

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ABSTRACT

Background and aims: Blood cholesterol levels are regulated by competing mechanisms of cholesterol synthesis, absorption and excretion. Plant sterols are natural constituents of plants, are not synthesized in humans, and serve as markers for cholesterol absorption. Ezetimibe lowers the intestinal absorption of cholesterol and plant sterols. We analyzed the associations of differences in cholesterol metabolism, in particular increased cholesterol absorption, and the occurrence of in-stent restenosis (ISR) in patients with stable coronary artery disease.

Methods: Elective stent implantation of de novo stenosis was conducted in 59 patients (74.6 % males, 67.2 ± 9.6 years). Cholesterol and non-cholesterol sterols were quantified in serum samples by gas chromatography or mass spectrometry. ISR was assessed by optical coherence tomography (OCT) and quantitative angiography (QCA) after six months.

Results: Markers for cholesterol absorption (e.g. campesterol-to-cholesterol) were positively associated with ISR measured by QCA (%diameter stenosis, late lumen loss) and OCT (proliferation volume, %area stenosis), whereas markers for cholesterol synthesis (e.g. lathosterol-to-cholesterol) were negatively associated with ISR (%area stenosis: r = −0.271, p = 0.043). There was no association between ISR and total cholesterol, LDL, HDL, triglycerides. Markers for cholesterol absorption (e.g. campesterol-to-cholesterol) were significantly lower in ezetimibe-treated patients compared to patients on a statin only (1.29 ± 0.69 vs. 2.22 ± 1.23; p = 0.007). Combined lipid-lowering with ezetimibe plus statin reduced ISR compared to statin only (13.7 ± 10.4 vs. 22.5 ± 12.1 %diameter stenosis, p = 0.015).

Conclusions: Differences in cholesterol metabolism, more specifically increased cholesterol absorption, are associated with ISR.

1. Introduction

In-stent restenosis (ISR) is one of the major limitations after successful coronary angioplasty despite modern drug-eluting stents [11]. The underlying pathophysiological mechanisms are (a) neointimal hyperplasia, which occurs secondary to migration and proliferation of vascular smooth muscle cells, and (b) development of neoatherosclerosis due to accumulation of extracellular matrix among others [2–4].

Mechanisms of ISR are complex and not yet fully understood. In vitro cell and in vivo animal studies suggest that these mechanisms are influenced by lipoproteins [2,5,6]. However no study has investigated the association of restenosis with a detailed analysis of cholesterol metabolism so far.

Serum cholesterol concentrations are derived from two entirely different sources: endogenous cholesterol from hepatic and extrahepatic synthesis, and exogenous cholesterol and plant sterols (so-
called xenosterols) derived from intestinal absorption of dietary and biliary sterols. Therefore, cholesterol homeostasis is reflected by the ratio of dietary cholesterol absorption to endogenous cholesterol synthesis [7]. Lathosterol, a precursor in cholesterol synthesis is a marker for hepatic synthesis rate. Serum concentrations of plant sterols, such as campesterol and sitosterol, which are solely of dietary origin, reflect the efficacy of cholesterol absorption. In healthy individuals, approximately 50 % of cholesterol is absorbed via the intestine [8]. This ratio is disturbed in patients with sitosterolemia [9]. This autosomal-recessive disease is characterized by increased absorption and diminished biliary elimination of dietary cholesterol and plant sterols caused by a defect in the ABCG5 or ABCG8 transporter genes leading to increased serum plant sterol levels, premature atherosclerosis, and early cardiovascular death [10]. The understanding of this disease brought up speculations that plant sterols „per se” are atherogenic. This hypothesis was verified only recently by Helgadottir and colleagues in a large cohort of healthy “non-sitosterolemic” individuals [11]. Using genetic risk scores (GRS) they found that variants which regulate intestinal cholesterol absorption accounted for 62 % of CAD risk regulated by non-HDL-cholesterol, but the remaining 38 % was due to increased absorption of plant sterols. Our group has reported previously that plant sterols impair endothelial function, increase cerebral ischemic lesions and aggravate atherosclerosis in mice [12]. In clinical studies plant sterol depositions were found in aortic valve cusps and in atherosclerotic lesions and, most importantly, plant sterol plasma levels were associated with hard cardiovascular clinical outcomes [12-14].

The aim of the present study was to assess whether differences in cholesterol metabolism and in particular increased cholesterol and plant sterol absorption (e.g. campesterol and sitosterol) affect the occurrence of in-stent proliferation and in-stent restenosis (ISR) in stable coronary artery disease.

2. Methods

2.1. Study design and patient population

We investigated a subset of data from the prospective, randomized Octopus trial (clinicaltrials.gov, NCT: 01056744), that compared two different drug eluting devices in a 1:1 randomization in patients with stable CAD and indication for elective PCI between 2009 and 2011. The details of the trial design and the main results have been published elsewhere [15,16]. Here, we focused on laboratory markers of cholesterol metabolism and its association with lipid-lowering therapy and the development of ISR. ISR was investigated clinically and during scheduled re-coronary angiography including optical coherence tomography (OCT, Time-domain OCT, M2 CV system, LightLab Imaging Inc., Westford, MA, USA) six months after PCI. All study participants gave written informed consent. The study was approved by the local ethics committee of the University Hospital of Jena (identification number: 2392–10/2008) and conducted according to the principles of the Declaration of Helsinki.

2.2. Coronary angiography and optical coherence tomography

All patients underwent re-coronary angiography including intracoronary imaging of the stented lesion by OCT six months after index PCI. Quantitative coronary angiography (QCA) was assessed offline by two observers according to the 15-coronary tree segment system (CAAS version 5.9.2, 2012, Pie Medical Imaging, Maastricht, Netherlands). Percent diameter stenosis, late lumen loss (LLL = final MLD at the end of the index procedure - MLD at follow-up) and net lumen gain (NLG = MLD at follow-up - baseline MLD) were calculated. OCT images of the stented coronary segment were analyzed frame by frame by a detailed OCT protocol [15,16]. In-stent proliferation area (=stent area – lumen area, mm²) and maximum in-stent proliferation thickness (mm) were measured for each frame. Volumetric parameters were computed through the integral of area measurements over stent length. Further parameters were calculated: (1) relative in-stent proliferation volume (%) = proliferation volume / analyzed stent length) * 100; (2) maximum and median relative proliferation area = (1 – (minimal lumen area/stent area within the same frame)) × 100, (3) in-stent area stenosis (%), (4) in-stent diameter stenosis (%).

2.3. Measurement of sterols and Oxy(phyto)sterols

Peripheral venous blood samples were drawn and frozen for later analysis at index hospitalization additionally to routine blood work according to the hospital’s standard operating procedures for total cholesterol (enzymatically), LDL- and HDL-cholesterol and triglycerides. Cholesterol, non-cholesterol sterols and oxy(phyto)sterols were quantified in serum samples. After alkaline hydrolysis, the free sterols and oxy (phyto)sterols were extracted with chloroform/methanol (2:1; v/v). Sterols were separated from oxy(phyto)sterols by silica Sep-Pak cartridges using solvents with increasing polarity. The trimethylsilylated (TMSi-) steroid and di-TMSi-oxy(phyto)sterol ethers were separated by gas chromatography (GC). Cholesterol/TMSi ethers were detected by less sensitive but specific flame-ionization detection (FID) (5α-cholestane, internal standard, ISTD), the non-cholesterol steroid-TMSi ethers (epicoprostanol-TMSi ether, ISTD) and the oxy(phyto)sterol di-TMSi ethers (5α-lcoxoxy(phyto)sterol di-TMSi ethers, ISTDs) by highly specific and highly sensitive mass spectrometry in the selected ion monitoring mode (MS-SIM) as described en detail previously [17-19].

2.4. Statistical analysis

All parameters were archived in a custom-made database (Microsoft Access, Microsoft Inc.). Statistical calculations were done with SPSS (version 24.0, IBM SPSS statistics). Continuous variables are expressed as mean ± SD and analyzed with the Student t-test for normal distribution. Categorical variables are presented as percent. Bivariate analysis with the Spearman correlation coefficient was used to determine the strength of relationship between cholesterol markers and parameters of ISR. Multiple linear regression analysis was done to test for potential confounding variables. Statistical significance was assumed for p-values < 0.05 (two-tailed).

3. Results

3.1. Clinical and procedural characteristics of the study population

The baseline characteristics of the patients enrolled in the study are shown in Table e1 (Supplement). The participants included 44 males (74.6%), 17 current smokers (28.8 %) and 26 (44.1%) patients with type 2 diabetes. Total serum cholesterol levels were on average 169.1 mg/dL. All but one patient (96.6 %) received lipid-lowering therapy, of which 15 (25.4 %) patients were treated additionally with ezetimibe.

3.2. Markers of cholesterol metabolism and lipid-lowering therapy at baseline

Marks of cholesterol absorption such as sitosterol, campesterol and cholesterol with their ratios to total cholesterol, as well as the ratio of campesterol-to-lathosterol were lower in ezetimibe treated patients (Table 2., Suppl. eFig. 1). On the other hand, cholesterol absorption inhibition by ezetimibe led to an upregulation of endogenous cholesterol synthesis depicted by significantly higher levels of the synthesis marker lathosterol and a trend for the lathosterol-to-cholesterol ratio (Table 2., Suppl. eFig. 1).

3.3. In-stent restenosis in statin vs. statin/ezetimibe treated patients

All interventions during index PCI were successful (residual %
good 6-month PCI result in the entire patient population we found 187.5. QCA, quantitative coronary angiography; OCT, optical coherence tomography. dissections). Patients were invasively followed-up after a mean of 27.7 days. Despite the relative small sample size and an overall < 20% during final angiography, no flow-limiting proliferation resulted in reduced ISR compared to patients on statins. We confirm that dietary sterol absorption (campesterol-to-cholesterol and sitosterol-to-cholesterol with %diameter stenosis in QCA ($r = 0.303; p = 0.049$ and $r = 0.332, p = 0.03$), and between the absorption markers campesterol-to-cholesterol, sitosterol-to-cholesterol and campesterol-to-lathosterol and relative proliferation volume (%) measured by OCT ($r = 0.412; p = 0.007; r = 0.402, p = 0.009$ and $r = 0.332, p = 0.034$). These associations were not detectable in the combined treatment group of statins and ezetimibe.

### 4. Discussion

A combined lipid lowering therapy with a statin and ezetimibe resulted in reduced ISR compared to patients on statins. We confirm that dietary sterol absorption (campesterol-to-cholesterol and campesterol-to-lathosterol-ratios) is positively associated with ISR in QCA and OCT-analysis, while cholesterol synthesis showed negative associations. On the contrary, conventional lipid risk factors such as total serum cholesterol, LDL- and HDL-cholesterol, and triglycerides were not associated with ISR.

Patients who were treated with a statin exhibited a positive correlation between in-stent restenosis and intestinal sterol absorption, whereas those treated with a statin and ezetimibe did not show any associations to in-stent proliferation. Moreover, in ezetimibe (a drug that inhibits NPC1L1 and reduces dietary cholesterol and plant sterol absorption) treated patients serum plant sterol levels were significantly reduced compared to those on statin monotherapy.

We believe that these results are of importance for a number of reasons. First, these results further add to the notion that plant sterols “per se” are atherogenic and that these sterols are also involved in vascular proliferative processes such as in-stent-restenosis. Second, a detailed analysis of markers of cholesterol metabolism prior to start of lipid-lowering therapy might identify patients at risk for ISR and cardiovascular events. This patient subset, so-called “hyper-absorbers” might benefit in particular by combined lipid-lowering therapy with a statin and ezetimibe.

### 4.1. Atherogenicity of plant sterols

Our group has previously demonstrated that plant sterols impaired endothelium-dependent vasodilatation and increased lesion size after cerebral ischemia and led to a more pronounced atherosclerotic lesion formation [12]. In a clinical study we found that patients consuming plant sterol-enriched margarine were characterized by increased plasma concentrations of plant sterols and, importantly, increased tissue deposition [12]. More importantly, we demonstrated only recently that the oxysterol 7α-hydroxycampesterol was associated with cardiovascular events in patients undergoing coronary angiography and Ceglaèrek and colleagues verified that plant sterol tissue depositions in plaques of carotid arteries are associated with ischemic stroke [14,20]. These data suggested a potential negative vascular effect, depending on plant sterol rather than plasma cholesterol concentrations. Genetic association studies in non-sitosterolemic humans support the aspect that plant sterols “per se” are atherogenic. Teupser and colleagues confirmed that mutations of the gene locus of ABCG5/8 co-transporters subsequently leading to increased plant sterol concentrations were associated with.

### Table 2

| Marker of Cholesterol metabolism | All patients | Patients on statin | Patients on statin/ezetimibe | $p$ |
|-------------------------------|-------------|-------------------|-----------------------------|-----|
|                               | $N = 59$    | $N = 57$          | $N = 15$                    |     |
| I. Absorption                 |             |                   |                             |     |
| Sitosterol (mg/dl)            | 0.19 ± 0.13 | 0.21 ± 0.12       | 0.16 ± 0.14                 | 0.155 |
| Campesterol (mg/dl)           | 0.33 ± 0.22 | 0.36 ± 0.19       | 0.27 ± 0.30                 | 0.188 |
| Cholestanol (mg/dl)           | 0.26 ± 0.10 | 0.26 ± 0.07       | 0.25 ± 0.14                 | 0.753 |
| Sitosterol-to-cholesterol     | 1.18 ± 0.80 | 1.33 ± 0.87       | 0.78 ± 0.34                 | 0.021 |
| Campesterol-to-cholesterol    | 2.0 ± 1.12  | 2.22 ± 1.23       | 1.29 ± 0.69                 | 0.007 |
| Cholestanol-to-cholesterol    | 1.54 ± 0.25 | 1.60 ± 0.23       | 1.37 ± 0.23                 | 0.002 |
| Campesterol-to-lathosterol    | 338.6 ± 276.2 | 387.1 ± 291.8   | 219.0 ± 178.7              | 0.041 |
| II. Synthesis                 |             |                   |                             |     |
| Lathosterol (mg/dl)           | 0.12 ± 0.09 | 0.11 ± 0.04        | 0.16 ± 0.15                 | 0.035 |
| Lathosterol-to-cholesterol    | 0.72 ± 0.33 | 0.66 ± 0.22       | 0.84 ± 0.50                 | 0.071 |

QCA, quantitative coronary angiography; OCT, optical coherence tomography.

### Table 3

| In-stent restenosis and in-stent proliferation at 6-months f/u in patients on statin vs. statin/ezetimibe therapy. |
|----------------------------------------------------------------------------------------------------------------|
|Patients on statins | Patients on statin/ezetimibe | $p$ |
|---------------------|-----------------------------|-----|
| $N = 57$            | $N = 15$                    |     |
| QCA                 |                             |     |
| Minimal lumen diameter (mm) | 2.01 ± 0.47 | 2.21 ± 0.39 | 0.155 |
| Late lumen loss (mm) | 0.23 ± 0.23                 | 0.12 ± 0.1 | 0.080 |
| Diameter stenosis (%) | 22.5 ± 12.1                  | 13.7 ± 10.4 | 0.015 |
| OCT                 |                             |     |
| Maximum diameter stenosis (%) | 64.2 ± 12.1                  | 56.3 ± 25.2 | 0.127 |
| Maximum area stenosis (%) | 42.2 ± 16.0                  | 37.6 ± 13.8 | 0.340 |
| Relative proliferation volume (%) | 23.1 ± 11.5                  | 21.8 ± 7.9 | 0.705 |

3.4. Markers of cholesterol metabolism and In-stent restenosis

We found no association between ISR assessed by QCA and OCT and conventional lipid risk factors (total cholesterol, LDL, HDL, triglycerides: Table 4., Suppl. Fig. 2). However, ISR measured by QCA and OCT was positively associated with various cholesterol absorption markers despite a relatively short-term f/u and a small sample size (Table 4., Fig. 2). Conversely, we found negative association for the cholesterol synthesis parameter lathosterol with its ratio to cholesterol and ISR (Table 4.).

In the group of patients treated with statins only we still found a positive association between the cholesterol absorption markers campesterol-to-cholesterol and sitosterol-to-cholesterol with %diameter stenosis in QCA ($r = 0.303; p = 0.049$ and $r = 0.332, p = 0.03$), and between the absorption markers campesterol-to-cholesterol, sitosterol-to-cholesterol and campesterol-to-lathosterol and relative proliferation volume (%) measured by OCT ($r = 0.412; p = 0.007; r = 0.402, p = 0.009$ and $r = 0.332, p = 0.034$). These associations were not detectable in the combined treatment group of statins and ezetimibe.
same conclusions as previous studies that increased plant sterols confer greater risk of CAD than predicted by their effect solely on non-HDL cholesterol [11]. They identified nine rare ABCG5/8 coding variants with substantial impact on plasma non-HDL cholesterol and phytosterols. A GRS of ABCG5/8 variants which predicted 1 mmol/L increase in non-HDL cholesterol was associated with a 2-fold increase in CAD risk. GRSs for other genes that regulate non-HDL cholesterol (such as APOB, HMG-reductase, PCSK9 and LDL receptor etc.) but which do not regulate plant sterol levels, predicted that a 1 mmol/L increase in non-HDL cholesterol increased the CAD risk by 1.5-fold. Helgadottir and colleagues concluded that variants in ABCG5/8 accounted for about 62% of CAD risk by regulating non-HDL cholesterol, but the remaining 38% was due to changes in plant sterol levels, concluding that plant sterols “per se” are atherogenic [11].

4.2. Cholesterol metabolism and lipid-lowering therapy

As early as 1989 a subgroup analysis of the Scandinavian Simvastatin Survival Study (4S) study revealed that patients with high cholesterol absorption did not benefit from statin treatment. In fact, in patients in the highest quartile of cholesterol absorption 1% lowering with simvastatin was associated with an increase in cardiovascular events [23]. These findings are of particular importance since statins have been shown to increase cholesterol and plant sterol absorption and it has been speculated that the increase in cardiovascular events has been attributed to increased absorption of plant sterols [24]. The recently published HJ-Proper Study can be regarded as a “proof of concept” [25]. In that study only patients with high plant sterol plasma levels – so-called “high absorbers” - demonstrated a significant reduction in cardiovascular events by the addition of ezetimibe, whereas those with low cholesterol absorption did not. Moreover, a recently published study in hypercholesterolemic patients could show the particular benefit of an ezetimibe-atorvastatin combination therapy in patients with a mutation in ABCG5/8 genes [26].

4.3. Cholesterol metabolism and atherosclerosis disease progression on IVUS and OCT

Several studies have demonstrated that serum cholesterol levels do not affect ISR [27,28]. Moreover, statin treatment did not affect the occurrence of restenosis neither after balloon angioplasty nor after stent implantation [29,30].

Clinical studies using intravascular imaging and analyzing cholesterol metabolism in more detail, however, offer more precise insights. Nasu and colleagues reported that increased cholesterol absorption (campesterol-to-lathosterol) was associated with atherosclerotic plaque vulnerability assessed by OCT and virtual histology [31]. Another IVUS-study found that hypo-responsiveness to statin treatment is associated with lipid profiles such as LDL cholesterol, HDL cholesterol, triglycerides, non-HDL cholesterol and other markers of vascular health [32].
with aggressive neointimal formation 12 months after stent implantation [32]. We suggested earlier to measure plant sterols in that study since statin hypo-responsiveness is balanced by increased plant sterol absorption [24,33].

Observations in GLAGOV (Global Assessment of Plaque ReGression with PCSK9 AntibOdy as Measured by IntraVascular Ultrasound) and PRECISE-IVUS (Plaque Regression With Cholesterol Absorption Inhibitor or Synthesis inhibitor Evaluated by IntraVascular UltraSound) fit into this pattern. Combined lipid-lowering with a statin and ezetimibe in PRECISE-IVUS resulted in a more pronounced atherosclerotic lesion regression compared to the results achieved in GLAGOV [34–36]. These differences cannot be explained by LDL-C lowering “per se” which was less effective by statin and ezetimibe treatment (73 mg/dl to 63 mg/dl) compared to a much more effective LDL-C lowering by statins and the PCSK9-inhibitor Evolucumab (93 mg/dl to 36 mg/dl). Subgroup analysis of PRECISE-IVUS further support this notion. Although there has been a close correlation between achieved LDL-C levels and the change in coronary atheroma volume consistently in prior IVUS trials, the plot is located far below the line in the atorvastatin/ezetimibe combination arm of the PRECISE-IVUS trial, suggesting a potential existence of a “beyond LDL-C-lowering effect” of statin/ezetimibe combination therapy. Comparable to our results progression of percent atheroma volume (PAV) was positively associated with plant sterol plasma levels and negatively associated with lathosterol a marker for endogenous cholesterol synthesis [37]. In a subsequent subgroup analysis of PRECISE-IVUS the effect of ezetimibe on serial change in PAV was more pronounced in patients with statin pretreatment. Furthermore, statin and ezetimibe combination therapy resulted in greater total atheroma volume (TAV) regression compared to patients on statin only [35]. The authors concluded, that the compensatory increase in cholesterol (and plant sterol) absorption in statin treated patients is associated with atherosclerotic disease progression. The findings of our study further add to the notion that increased cholesterol – and in particular plant sterol absorption – has detrimental effects in vascular proliferative disorders such as atherosclerosis and restenosis.

5. Limitations

The sample size of our study is relatively small and data were analyzed post-hoc. The lipid-lowering therapy groups statin vs. statin/ezetimibe were purely observational and clinically initiated. Moreover, we were investigating surrogate parameters since this sub-study was not designed for clinical endpoints.

6. Conclusion

We demonstrate that differences in cholesterol metabolism, more specifically increased dietary cholesterol and plant sterol absorption, increase in-stent-restenosis. These findings add to the concept of individualized lipid-lowering therapy and have to be verified in future prospective clinical trials [38].

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.steroids.2022.109079.

References

[1] F. Alfonso, R.A. Byrne, F. Rivero, A. Kastrati, Current treatment of in-stent restenosis, J Am Coll Cardiol. 63 (24) (2014) 2659–2673.
[2] M. Nakano, R. Virmani, Histopathology of vascular response to drug-eluting stents: An insight from human autopsy into daily practice, Cardiovascular intervention and therapeutics. 30 (1) (2015) 1–11.
[3] G. Nakazawa, F. Otsuka, M. Nakano, M. Vorpahl, S.K. Yazdani, E. Ladich, F.D. Kolodgie, A.V. Finn, R. Virmani, The pathology of neoatherosclerosis in human coronary implants bare-metal and drug-eluting stents, J Am Coll Cardiol. 57 (11) (2011) 1314–1322.
[4] M. Oberhofer, C. Herdeg, A. Baumbach, K. Shamat, A. Kranzhofer, O. Weingartner, K. Rührman, M. Kluge, K.R. Karsch, Time course of smooth muscle cell proliferation after local drug delivery of low-molecular-weight heparin using a porous balloon catheter, Cathet. Cardiovasc. Diagn. 41 (3) (1997) 268–274.
[5] K. Skälen, M. Gustafsson, E.K. Rydberg, L.M. Hultén, O. Wiklund, T.L. Innerarity, J. Boren, Subendothelial retention of atherogenic lipoproteins in early atherosclerosis, Nature 417 (6890) (2002) 750–754.

Fig. 2. Cholesterol absorption (e.g. campesterol-to-lathosterol-ratio) is associated with the occurrence of In-stent restenosis (ISR) and In-stent proliferation assessed by QCA (left) OCT (right).
A. Kerksiek, S. Friedrichs, U. Ulbricht, A.M. Zawada, U. Laufs, B. Scheller, D. Fliser, P. C. Schulze, M. Bohm, G. H. Heine, D. Lütjohann, Plasma levels of the human serum, Chem Phys Lipids. 164 (6) (2011) 425

J Steroid Biochem Mol Biol. 190 (2019) 1

S. Lander, E. Boerwinkle, S. Gabriel, S. Kathiresan, Inactivating mutations in NPC1L1 and protection from coronary heart disease, N Engl J Med. 371 (2014) 1346.

K. Nakao, T. Tobaru, H. Tanaka, T. Oka, Y. Endo, K. Saito, T. Uchida, K. Matsui, H. Ogawa, N. Hagiwara, Baseline serum sitosterol level as predictor of adverse clinical events in acute coronary syndrome patients with dyslipidemia: A sub-analysis of hij-proper, Atherosclerosis. 274 (2018) 139–145.

H. Tada, H. Okada, A. Nomura, M. Takamasa, M. Kawashita, Effect of ezetimibe-atorvastatin combination therapy in patients with a mutation in ABCG5 or ABCG8 gene, Lipids Health Dis. 19 (2020) 3.

T.A. Miettinen, M. Alaka, M. Lepantalo, H. Ylihull, Plant sterols in serum and in atherothrombotic plaques of patients undergoing carotid endarterectomy, J Am Coll Cardiol. 45 (11) (2005) 1794–1801.

K. Tsujita, S. Sugiyama, H. Sumida, H. Shimomura, T. Yamashita, K. Yamanaga, N. Komura, K. Sakamoto, S. Sugiyama, H. Sumida, K. Nasu, M. Terashima, M. Habara, E. Ko, T. Ito, D. Yokota, S. Ishizuka, T. Kurita, M. Kimura, Y. Kinoshita, Y. Asaka, T. Matsui, H. Suzuki, Impact of cholesterol metabolism on coronary plaque vulnerability of target vessels: A combined analysis of virtual histology intravascular ultrasound and optical coherence tomography, JACC Cardiovasc Interv. 6 (2013) 746–755.

Y. U. Kataoka, J. St. John, K. Wolski, K. Uno, P. E. Turz, S. E. Nissen, S. J. Nichols, Atheroma progression in hyperresponders to statin therapy, Arterioscler Thromb Vasc Biol. 35 (4) (2015) 990–995.

T. Weingartner O, Lütjohann D, Elsasser A, Lauts U, Personalized lipid-lowering therapy to further reduce residual cardiovascular risk. Arterioscler Thromb Vasc Biol. 2015; ATVB04A114.304477.

S. J. Nichols, R. Puri, T. Anderson, C. M. Ballantyne, L. Cho, J. J. Kastelein, W. Koenig, R. Somarasekare, H. Kasusajan, J. Yang, S. M. Wasserman, R. Scott, I. Ungi, J. Podolec, A. O. Ophuls, J. H. Correa, M. Borgerding, D. M. Goodman, S. E. Nissen, Effect of evolocumab on progression of coronary disease in statin-treated patients: The glagov randomized clinical trial, JAMA, J. Am. Med. Assoc. 316 (2016) 2373–2384.

K. Tsujita, K. Yamanaka, N. Komura, K. Sakamoto, S. Sugiyama, H. Sumida, H. Shimomura, T. Yamashita, H. Oka, K. Nakao, S. Nakamura, M. Ishihara, K. Matsui, N. Sakai, N. Nakano, N. Yamanaka, Y. Asaka, T. Matsui, K. Fujimoto, R. Tsunoda, Y. Morikami, K. Matsuura, S. Oshima, K. Kaikita, S. Hokimoto, H. Ogawa, P. Investigators, Synergistic effect of ezetimibe addition on coronary atheroma regression in patients with prior statin therapy: Subanalysis of precise-ivus trial, Eur J Prev Cardiol. 23 (2016) 1524–1528.

K. Tsujita, S. Sugiyama, H. Sumida, H. Shimomura, T. Yamashita, K. Yamanaka, N. Komura, K. Sakamoto, H. Oka, K. Nakao, S. Nakamura, M. Ishihara, K. Matsui, N. Sakai, N. Nakano, N. Yamanaka, Y. Asaka, T. Matsui, K. Fujimoto, R. Tsunoda, Y. Morikami, K. Matsuura, S. Oshima, K. Kaikita, S. Hokimoto, H. Ogawa, P. Investigators, Synergistic effect of ezetimibe addition on coronary atheroma regression in patients with prior statin therapy: Subanalysis of precise-ivus trial, Eur J Prev Cardiol. 23 (2016) 1524–1528.

K. Tsujita, K. Yamanaka, N. Komura, K. Sakamoto, S. Sugiyama, H. Sumida, H. Shimomura, T. Yamashita, H. Oka, K. Nakao, S. Nakamura, M. Ishihara, K. Matsui, N. Sakai, N. Nakano, N. Yamanaka, Y. Asaka, T. Matsui, K. Fujimoto, R. Tsunoda, Y. Morikami, K. Matsuura, S. Oshima, K. Kaikita, S. Hokimoto, H. Ogawa, P. Investigators, Synergistic effect of ezetimibe addition on coronary atheroma regression in patients with prior statin therapy: Subanalysis of precise-ivus trial, Eur J Prev Cardiol. 23 (2016) 1524–1528.

K. Tsujita, K. Yamanaka, N. Komura, K. Sakamoto, S. Sugiyama, H. Sumida, H. Shimomura, T. Yamashita, H. Oka, K. Nakao, S. Nakamura, M. Ishihara, K. Matsui, N. Sakai, N. Nakano, N. Yamanaka, Y. Asaka, T. Matsui, K. Fujimoto, R. Tsunoda, Y. Morikami, K. Matsuura, S. Oshima, K. Kaikita, S. Hokimoto, H. Ogawa, P. Investigators, Synergistic effect of ezetimibe addition on coronary atheroma regression in patients with prior statin therapy: Subanalysis of precise-ivus trial, Eur J Prev Cardiol. 23 (2016) 1524–1528.

K. Tsujita, K. Yamanaka, N. Komura, K. Sakamoto, S. Sugiyama, H. Sumida, H. Shimomura, T. Yamashita, H. Oka, K. Nakao, S. Nakamura, M. Ishihara, K. Matsui, N. Sakai, N. Nakano, N. Yamanaka, Y. Asaka, T. Matsui, K. Fujimoto, R. Tsunoda, Y. Morikami, K. Matsuura, S. Oshima, K. Kaikita, S. Hokimoto, H. Ogawa, P. Investigators, Synergistic effect of ezetimibe addition on coronary atheroma regression in patients with prior statin therapy: Subanalysis of precise-ivus trial, Eur J Prev Cardiol. 23 (2016) 1524–1528.