Biochemical and Biophysical Research Communications 407 (2011) 153–157

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Chronic administration of ezetimibe increases active glucagon-like peptide-1 and improves glycemic control and pancreatic beta cell mass in a rat model of type 2 diabetes

Soo Jin Yang [a], Jung Mook Choi [a], Lisa Kim [a], Byung-Joon Kim [b], Jin Hee Sohn [c], Won Jun Kim [d], Se Eun Park [d], Eun Jung Rhee [d], Won Young Lee [d], Ki Won Oh [d], Sung Woo Park [d], Sun Woo Kim [d], Cheol-Young Park [d,*]

[a] Diabetes Research Institute, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea
[b] Division of Endocrinology, Department of Internal Medicine, Konyang University, School of Medicine, Daejeon, Republic of Korea
[c] Department of Pathology, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea
[d] Division of Endocrinology and Metabolism, Department of Internal Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul 110-746, Republic of Korea

ARTICLE INFO

Article history:
Received 21 February 2011
Available online 1 March 2011

Keywords:
Ezetimibe
Glucagon-like peptide-1
Glycemic control
Pancreatic beta cell
Type 2 diabetes

ABSTRACT

Ezetimibe is a cholesterol-lowering agent targeting Niemann-Pick C1-like 1, an intestinal cholesterol transporter. Inhibition of intestinal cholesterol absorption with ezetimibe may ameliorate several metabolic disorders including hepatic steatosis and insulin resistance. In this study, we investigated whether chronic ezetimibe treatment improves glycemic control and pancreatic beta cell mass, and alters levels of glucagon-like peptide-1 (GLP-1), an incretin hormone involved in glucose homeostasis. Male LETO and OLETF rats were treated with vehicle or ezetimibe (10 mg kg^{-1} day^{-1}) for 20 weeks via stomach gavage. OLETF rats were diabetic with hyperglycemia and significant decreases in pancreatic size and beta cell mass compared with LETO lean controls. Chronic treatment of OLETF rats with ezetimibe improved glycemic control during oral glucose tolerance test compared with OLETF controls. Moreover, ezetimibe treatment rescued the reduced pancreatic size and beta cell mass in OLETF rats. Interestingly, ezetimibe significantly decreased serum dipeptidyl peptidase-4 activity and increased serum active GLP-1 in OLETF rats without altering serum total GLP-1. These findings demonstrated that chronic administration of ezetimibe improves glycemic control and pancreatic beta cell mass, and increases serum active GLP-1 levels, suggesting possible involvement of GLP-1 in the ezetimibe-mediated beneficial effects on glycemic control.

1. Introduction

Hypercholesterolemia is a condition that often aggravates diabetes and its complications [1,2]. It has been suggested that high levels of cholesterol in circulation and tissues are pro-inflammatory mediators and that reducing cholesterol levels can be a therapeutic strategy for inflammation-mediated metabolic disorders including insulin resistance, hepatic steatosis, and non-alcoholic steatohepatitis (NASH) [3,4].

Ezetimibe selectively inhibits intestinal cholesterol absorption by blocking Niemann-Pick C1-like 1 (NPC1L1) [5,6]. In humans, ezetimibe has been used as a monotherapy or in combination with statins to manage hyperlipidemia. Previous studies reported that inhibition of intestinal cholesterol absorption with ezetimibe may ameliorate several metabolic disorders including hepatic steatosis and insulin resistance [4,7–10]. However, research is limited with regard to insulin resistance, and an underlying molecular mechanism has not been revealed.

Glucagon-like peptide-1 (GLP-1), an incretin hormone secreted from intestinal L-cells in response to nutrient ingestion, regulates glucose homeostasis by altering secretion of pancreatic hormones including insulin and glucagon [11–13]. GLP-1 is rapidly inactivated by dipeptidyl peptidase-4 (DPP-4)-mediated cleavage. High DPP-4 activity and low levels of active GLP-1 were observed in diabetic patients [14,15]. In contrast, administration of DPP-4 inhibitor increased release of GLP-1 and other incretin hormones, which subsequently improved pancreatic beta cell functions and glycemic control [16–18].

In the present work, we investigated the effects of chronic ezetimibe treatment on glycemic control, pancreatic beta cell mass

Abbreviations: ABCG5, ATP binding cassette transporters G5; DPP-4, dipeptidyl peptidase-4; FFA, free fatty acids; GLP-1, glucagon-like peptide-1; NASH, non-alcoholic steatohepatitis; NPC1L1, Niemann-Pick C1-like 1; TC, total cholesterol; TG, triglyceride.

* Corresponding author. Fax: +82 2 2001 1588.
E-mail address: cydoctor@chol.com (C.-Y. Park).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.02.129

and GLP-1 levels. Our data demonstrated that chronic ezetimibe administration improved glycemic control and pancreatic beta cell mass. The improvement was accompanied by alterations in active GLP-1 levels and DPP-4 activity, suggesting that GLP-1 may play a role in the ezetimibe-mediated beneficial effects.

2. Materials and methods

2.1. Animals

Male OLETF rats and age-matched LETO (lean control) rats were provided by Otsuka Pharmaceutical (Tokushima, Japan). Rats were maintained in a temperature- and humidity-controlled room with a 12 h light/dark cycle and fed PicoLab Rodent Diet 20 5053 (5% wt/wt fat; Purina Mills, Richmond, IN, USA) with unlimited access to food and water. A total of 19 rats at approximately 12 weeks of age were treated with ezetimibe (10 mg kg^{-1} day^{-1}) or with PBS as a control vehicle via stomach gavage for 20 weeks. The study protocol conformed to the specifications outlined in the National Institutes of Health's Guiding Principles for the Care and Use of Laboratory Animals and was approved by the Institutional Animal Care and Use Committee of the Sungkyunkwan University Kangbuk Samsung Hospital.

2.2. Oral glucose tolerance test (OGTT)

OGTT was performed on rats before and after 6, 12, 16, and 20 weeks of treatment. After 16 h fasting, glucose solutions were given via stomach gavage (2 g kg^{-1}). Blood glucose levels were measured at 0, 15, 30, 60, 90 and 120 min after the glucose challenge using a Glucocard X-Meter (Arkray, Kyoto, Japan).

2.3. Blood and tissue collection

After 20 weeks of treatment, rats were anesthetized with intraperitoneal Zoletil/Rompun after an overnight fast and blood was collected from the abdominal aorta. After blood collection, tissues were harvested, weighed, and stored at −80 °C until further analysis.

2.4. Metabolic parameters

Glucose and triglyceride (TG) concentrations were measured by enzymatic assays (Sigma–Aldrich, St. Louis, MO, USA). Free fatty acids (FFA) concentrations were measured using a commercial kit from Wako (Wako Pure Chemical Industries, Osaka, Japan). Total cholesterol (TC) concentrations were measured using a cholesterol assay kit (Cayman Chemical Company, Ann Arbor, MI, USA). DPP-4 activities were measured using a commercial kit from Cayman. Commercially available ELISA kits were used for the measurement of adiponectin (B-Bridge International, Mountain View, CA, USA), insulin (Crystal Chem Inc., Downers Grove, IL, USA) and active GLP-1 (Millipore, Billerica, MA, USA).

2.5. Western blot analysis

Western blotting was performed as described previously [19]. Membranes were incubated with primary antibodies for GLP-1 (Abcam, Cambridge, UK) and β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Membranes were then exposed to an anti-rabbit secondary antibody conjugated to horseradish peroxidase (Santa Cruz Biotechnology). Signals were detected by chemiluminescence using the ECL detection reagent (GE Healthcare, Piscataway, NJ, USA). The bands were scanned by a Geliance 600 Imaging System (Perkin–Elmer, Waltham, MA, USA) with a cooled 12-bit camera, and quantified by densitometry. Levels of total GLP-1 were normalized to values for β-actin.

2.6. Immunohistochemistry and beta cell mass quantification

Sections (5 μm) of pancreases were prepared and immunohistochemistry was performed as described previously [20]. Beta cells in pancreas sections were identified using monoclonal anti-insulin antibody (1:1000 dilution; Sigma–Aldrich). For beta cell quantification, insulin-positive islet beta cells and total islet cells were counted using the ImageJ software program (available at http://rsb.info.nih.gov/ij/), and beta cell mass was expressed as a percentage of total islet cells.

2.7. Statistical analysis

All statistical analyses were performed using PASW Statistics 18 (SPSS, Chicago, IL, USA). Data are expressed as mean ± SEM. Student's *t* test was performed to compare two groups. One-way ANOVA was used when more than two groups were compared, and significance of observed differences among the groups was evaluated with a least significant difference post hoc test. Pearson's correlation coefficients (*r*) were used to describe the linear association between variables. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Chronic administration of ezetimibe improved glycemic control in OLETF rats

Weight changes were similar in all groups and food intake was not altered by ezetimibe treatment (Table 1). However, chronic ezetimibe treatment induced significant changes in the tissue weights of liver, kidney, and pancreas, but not in those of heart and fat pads (epididymal and subcutaneous fat pads) in OLETF rats (Table 1). Weights of liver and kidney in ezetimibe-treated OLETF rats decreased, which suggests the improvement of organomegaly in liver and kidney (Table 1). Reduced pancreatic size in OLETF rats was rescued to the levels of LETO lean controls with ezetimibe treatment (Table 1).

Table 1
Effects of ezetimibe on body weights, food intake and tissue weights.

	LETO control ($n = 6$)	OLETF control ($n = 6$)	OLETF ezetimibe ($n = 7$)
Body weight			
Baseline (g)	358 ± 5[a]	466 ± 6[b]	466 ± 7[b]
Post-treatment (g)	523 ± 5[a]	626 ± 18[b]	625 ± 7[b]
Weight change (g)	165 ± 7	160 ± 17	158 ± 11
Food intake (g/day)	24.4 ± 0.9[a]	37.7 ± 2.2[b]	37.5 ± 0.6[b]
Tissue weights (%)			
Liver	2.5 ± 0.1[a]	3.5 ± 0.2[b]	2.9 ± 0.1[a]
Heart	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Kidney	0.5 ± 0.0[a]	0.7 ± 0.1[b]	0.6 ± 0.0[a]
Pancreas	0.2 ± 0.1[ab]	0.1 ± 0.0[a]	0.2 ± 0.0[b]
Fat pads weights (%)			
Epididymal fat	1.3 ± 0.1[a]	2.6 ± 0.1[b]	2.4 ± 0.1[b]
Subcutaneous fat	1.1 ± 0.2[a]	2.7 ± 0.2[b]	2.6 ± 0.2[b]

Data are expressed as mean ± SEM ($n = 6$–7 per group). Tissue weights are expressed as a percentage of fasted body weight. Different letters within a variable are significantly different at $p < 0.05$.

OLETF rats were diabetic with hyperglycemia and hyperinsulinemia (Table 2). In addition, serum adiponectin concentrations were low and serum levels of lipids including FFA, TG, and TC were high in OLETF controls compared with lean controls (Table 2). Ezetimibe treatment significantly improved glycemic control (Fig. 1A) and all tested metabolic parameters (Table 2) in OLETF rats.

3.2. Ezetimibe treatment increased serum active GLP-1 levels in OLETF rats

To test whether and how ezetimibe alters active GLP-1, serum levels of active GLP-1 and DPP-4 activities were analyzed. The tendency of reduced GLP-1 levels in OLETF rats (Fig. 1C) was accompanied with significantly high levels of serum DPP-4 activity (Fig. 1B). Chronic ezetimibe treatment in OLETF rats induced a significant increase in serum active GLP-1 compared with OLETF controls (Fig. 1C). However, total GLP-1 was not altered by ezetimibe treatment (data not shown). Remarkably, there was significant reduction of serum DPP-4 activities in ezetimibe-treated OLETF rats (Fig. 1B), suggesting that alterations in serum GLP-1 by ezetimibe are probably due to altered serum DPP-4 activities.

A correlation analysis was conducted with Pearson's correlation coefficients (r) using data from OLETF rats. Serum active GLP-1 was negatively associated with OGTT area under the curve (AUC) ($r = -0.766$, $p = 0.004$) or tended to be negatively associated with serum glucose ($r = -0.559$, $p = 0.059$) and serum TC ($r = -0.556$, $p = 0.060$). Serum DPP-4 activity was positively associated with serum levels of glucose ($r = 0.877$, $p < 0.001$), insulin ($r = 0.744$, $p = 0.006$), FFA ($r = 0.776$, $p = 0.003$), TG ($r = 0.599$, $p = 0.040$), and TC ($r = 0.805$, $p = 0.002$). Serum DPP-4 activity was negatively associated with serum adiponectin ($r = -0.897$, $p < 0.001$), and tended to be positively associated with OGTT AUC ($r = 0.553$, $p = 0.062$).

3.3. Ezetimibe treatment increased pancreatic beta cell mass in OLETF rats

The effects of chronic ezetimibe treatment on pancreatic islets and islet beta cell mass were evaluated at the end of the study. Histological morphology showed that LETO pancreases were regular in shape; however, the pancreases of OLETF rats were irregular in shape and size. Ezetimibe administration to OLETF rats resulted in normal pancreas morphology with regular shape and more consistent size (Fig. 2). Pancreatic beta cell mass was low in diabetic OLETF rats compared with lean controls, which may explain the reduced pancreatic size in OLETF rats. OLETF rats chronically treated with ezetimibe manifested significantly restored beta cell mass compared with untreated OLETF rats (Fig. 2).

Table 2
Effects of ezetimibe on serum metabolic parameters.

	LETO control ($n = 6$)	OLETF control ($n = 6$)	OLETF ezetimibe ($n = 7$)
Glucose (mmol/l)	5.6 ± 0.8[a]	10.4 ± 0.2[b]	7.2 ± 0.1[c]
Insulin (pmol/l)	27.1 ± 3.9[a]	59.9 ± 4.1[b]	37.3 ± 0.9[c]
Free fatty acids (mmol/l)	0.4 ± 0.0[a]	0.7 ± 0.0[b]	0.5 ± 0.0[a]
Triglycerides (mmol/l)	5.5 ± 0.4[a]	13.9 ± 2.1[b]	9.6 ± 1.0[c]
Total cholesterol (μmol/l)	108.1 ± 7.1[a]	468.9 ± 14.6[b]	260.1 ± 6.6[c]
Adiponectin (μg/ml)	2.6 ± 0.1[a]	0.8 ± 0.1[b]	2.3 ± 0.1[c]

Data are expressed as mean ± SEM ($n = 6$–7 per group). Different letters within a variable are significantly different at $p < 0.05$.

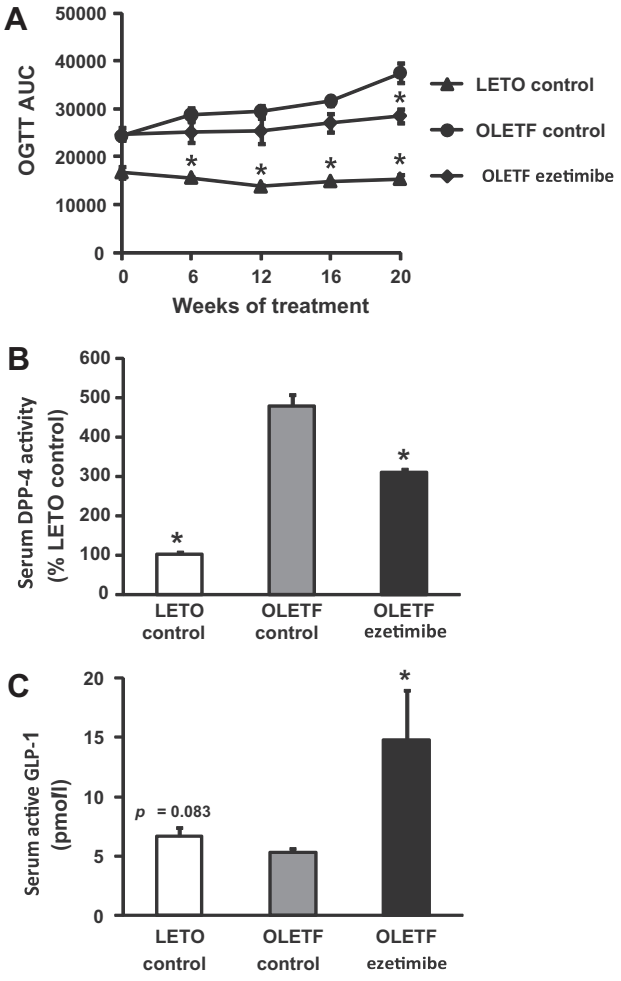

Fig. 1. Effects of ezetimibe treatment on glycemic control, serum dipeptidyl peptidase-4 (DPP-4) activities and serum levels of active glucagon-like peptide-1 (GLP-1). (A) Oral glucose tolerance test (OGTT) was performed before and after 6, 12, 16, and 20 weeks of treatment and area under the curve (AUC) is shown. (B) Serum DPP-4 activities. (C) Serum levels of active GLP-1. Data are mean ± SEM ($n = 6$–7 per group). *$p < 0.05$ compared with OLETF controls.

4. Discussion

Inhibition of cholesterol absorption with ezetimibe has been shown to have beneficial effects on metabolic regulations involved in glucose and lipid homeostasis [4,7–10]. Here, we report improved glycemic control accompanied by restoration of pancreatic size and islet beta cell mass with chronic ezetimibe treatment in a rat model of type 2 diabetes. Moreover, there were close correlations between serum active GLP-1 and OGTT AUC, as well as between serum DPP-4 activity and serum glucose, insulin and tested lipid parameters, suggesting that alterations in active GLP-1 and DPP-4 activity may be associated with the ezetimibe-mediated improvement in glycemic control.

Hypercholesterolemia often accompanies diabetes and is a condition that aggravates the development of diabetes and other related complications [1,2]. In apoE-deficient mice, hypercholesterolemia resulted in increased islet cholesterol concentrations and suppressed the insulin secretion capacity of islets [1]. Conversely, reducing cholesterol levels using cholesterol lowering agents improved hepatic steatosis and NASH in humans [7,8]. In a rodent model, a cholesterol-deficient diet ameliorated hepatic inflammation [3], and administration of cholesterol lowering agents improved metabolic disorders including insulin resistance,

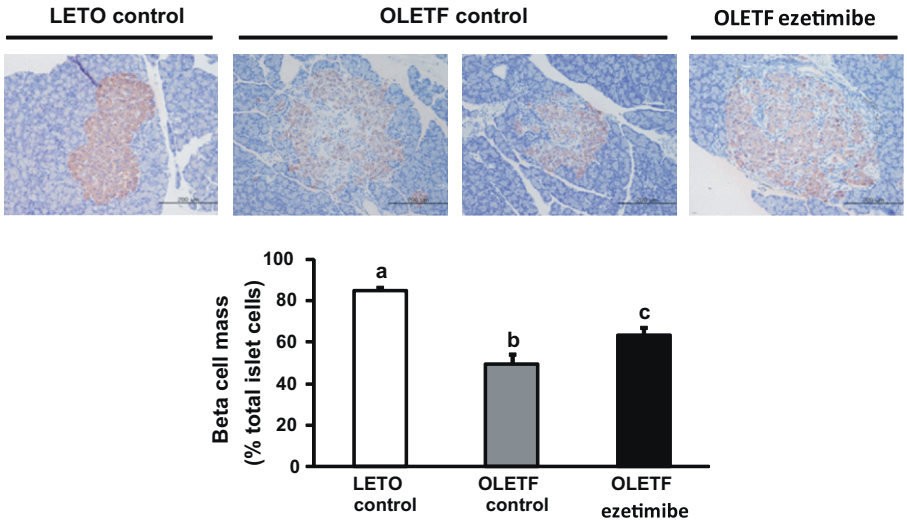

Fig. 2. Immunohistochemical analysis and quantification of pancreatic beta cell mass. Pancreatic sections from all the groups were stained with anti-insulin antibody. Representative islet images with insulin (red) staining. Insulin-positive beta cells were counted and beta cell mass was expressed as the percentage of beta cells relative to total islet cells. Original magnification, 200×. Data are mean ± SEM (n = 6–7 per group). Different letters within a variable are significantly different at $p < 0.05$. (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)

hepatic steatosis and non-alcoholic fatty liver disease [4,9]. Research has suggested that dysregulated cholesterol metabolism is present in diabetic patients [21,22]. Gene expression analysis of duodenal biopsy specimens from diabetic and non-diabetic patients showed up-regulated expression of *NPC1L1* and down-regulated expression of ATP binding cassette transporters G5 (*ABCG5*) and *ABCG8* in intestines from diabetic patients [21]. In a cross-sectional metabolic study in obese individuals, the rate of cholesterol synthesis was high in the diabetic group compared with controls and was significantly correlated with blood glucose concentrations [22], implying that diabetic conditions may modulate cholesterol metabolism. Together, these findings strongly suggested a link between glucose and cholesterol metabolism.

In the present study, chronic administration of ezetimibe was effective in glycemic control. In addition, we demonstrated that active GLP-1 was increased with ezetimibe treatment, which was accompanied by decreased DPP-4 activity. From our findings, it is not clear whether ezetimibe alters GLP-1 directly by acting on or binding with GLP-1 or whether the alteration in GLP-1 is secondary to other related changes (e.g. alterations in DPP-4 activity) in response to ezetimibe. Measurement of secreted GLP-1 levels in response to ezetimibe treatment in intestinal L-cells will help us to understand whether the ezetimibe-mediated alteration in GLP-1 is direct or indirect. It will also be very interesting to determine whether the altered GLP-1 is functionally involved in ezetimibe-mediated beneficial effects and whether other incretins also play a role in these effects.

In conclusion, the results of the current study demonstrate that chronic ezetimibe treatment is effective in improvement of glycemic control. The beneficial effect of ezetimibe on glycemic control is correlated with active GLP-1 levels. Our study shows ezetimibe-mediated improvement in pancreatic beta cell mass and possible involvement of GLP-1 in the ezetimibe-mediated effects. The findings reported here will help us to better understand metabolic regulation of ezetimibe and to develop more effective interventions for the prevention and treatment of diabetes.

Conflict of interest statement

None declared.

Acknowledgment

This study was supported by Samsung Biomedical Research Institute Grant, SBRI C-B1-111-1.

References

[1] M. Hao, W.S. Head, S.C. Gunawardana, A.H. Hasty, D.W. Piston, Direct effect of cholesterol on insulin secretion: a novel mechanism for pancreatic beta-cell dysfunction, Diabetes 56 (2007) 2328–2338.
[2] A. von Eckardstein, R.A. Sibler, Possible contributions of lipoproteins and cholesterol to the pathogenesis of diabetes mellitus type 2, Curr. Opin. Lipidol. 22 (2011) 26–32.
[3] K. Wouters, P.J. van Gorp, V. Bieghs, M.J. Gijbels, H. Duimel, D. Lutjohann, A. Kerksiek, R. van Kruchten, N. Maeda, B. Staels, M. van Bilsen, R. Shiri-Sverdlov, M.H. Hofker, Dietary cholesterol, rather than liver steatosis, leads to hepatic inflammation in hyperlipidemic mouse models of nonalcoholic steatohepatitis, Hepatology 48 (2008) 474–486.
[4] M. Deushi, M. Nomura, A. Kawakami, M. Haraguchi, M. Ito, M. Okazaki, H. Ishii, M. Yoshida, Ezetimibe improves liver steatosis and insulin resistance in obese rat model of metabolic syndrome, FEBS Lett. 581 (2007) 5664–5670.
[5] H.R. Davis, E.P. Veltri, Zetia: inhibition of Niemann-Pick C1 Like 1 (NPC1L1) to reduce intestinal cholesterol absorption and treat hyperlipidemia, J. Atheroscler. Thromb. 14 (2007) 99–108.
[6] M. Garcia-Calvo, J. Lisnock, H.G. Bull, B.E. Hawes, D.A. Burnett, M.P. Braun, J.H. Crona, H.R. Davis Jr., D.C. Dean, P.A. Detmers, M.P. Graziano, M. Hughes, D.E. Macintyre, A. Ogawa, A. O'Neill K, S.P. Iyer, D.E. Shevell, M.M. Smith, Y.S. Tang, A.M. Makarewicz, F. Ujjainwalla, S.W. Altmann, K.T. Chapman, N.A. Thornberry, The target of ezetimibe is Niemann-Pick C1-like 1 (NPC1L1), Proc. Natl. Acad. Sci. USA 102 (2005) 8132–8137.
[7] D.C. Chan, G.F. Watts, S.K. Gan, E.M. Ooi, P.H. Barrett, Effect of ezetimibe on hepatic fat, inflammatory markers, and apolipoprotein B-100 kinetics in insulin-resistant obese subjects on a weight loss diet, Diabetes Care 33 (2010) 1134–1139.
[8] M. Yoneda, K. Fujita, Y. Nozaki, H. Endo, H. Takahashi, K. Hosono, K. Suzuki, H. Mawatari, H. Kirikoshi, M. Inamori, S. Saito, T. Iwasaki, Y. Terauchi, K. Kubota, S. Maeyama, A. Nakajima, Efficacy of ezetimibe for the treatment of non-alcoholic steatohepatitis: an open-label, pilot study, Hepatol Res 40 (2010) 613–621.
[9] S. Zheng, L. Hoos, J. Cook, G. Tetzloff, H. Davis Jr., M. van Heek, J.J. Hwa, Ezetimibe improves high fat and cholesterol diet-induced non-alcoholic fatty liver disease in mice, Eur. J. Pharmacol. 584 (2008) 118–124.
[10] M. Nomura, H. Ishii, A. Kawakami, M. Yoshida, Inhibition of hepatic Neiman-Pick C1-like 1 improves hepatic insulin resistance, Am. J. Physiol. Endocrinol. Metab. 297 (2009) E1030–E1038.
[11] D.J. Drucker, The biology of incretin hormones, Cell Metab. 3 (2006) 153–165.
[12] D.J. Drucker, M.A. Nauck, The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes, Lancet 368 (2006) 1696–1705.
[13] P.L. Brubaker, The glucagon-like peptides: pleiotropic regulators of nutrient homeostasis, Ann. NY Acad. Sci. 1070 (2006) 10–26.

[14] E. Mannucci, L. Pala, S. Ciani, G. Bardini, A. Pezzatini, I. Sposato, F. Cremasco, A. Ognibene, C.M. Rotella, Hyperglycaemia increases dipeptidyl peptidase IV activity in diabetes mellitus, Diabetologia 48 (2005) 1168–1172.

[15] J. Ryskjaer, C.F. Deacon, R.D. Carr, T. Krarup, S. Madsbad, J. Holst, T. Vilsboll, Plasma dipeptidyl peptidase-IV activity in patients with type-2 diabetes mellitus correlates positively with HbAlc levels, but is not acutely affected by food intake, Eur. J. Endocrinol. 155 (2006) 485–493.

[16] V. Fonseca, A. Schweizer, D. Albrecht, M.A. Baron, I. Chang, S. Dejager, Addition of vildagliptin to insulin improves glycaemic control in type 2 diabetes, Diabetologia 50 (2007) 1148–1155.

[17] N. Mikhail, Incretin mimetics and dipeptidyl peptidase 4 inhibitors in clinical trials for the treatment of type 2 diabetes, Expert Opin. Investig. Drugs 17 (2008) 845–853.

[18] J.A. Pospisilik, J. Martin, T. Doty, J.A. Ehses, N. Pamir, F.C. Lynn, S. Piteau, H.U. Demuth, C.H. McIntosh, R.A. Pederson, Dipeptidyl peptidase IV inhibitor treatment stimulates beta-cell survival and islet neogenesis in streptozotocin-induced diabetic rats, Diabetes 52 (2003) 741–750.

[19] S.J. Yang, C.L. Keen, L. Lanoue, R.B. Rucker, J.Y. Uriu-Adams, Low nitric oxide: a key factor underlying copper-deficiency teratogenicity, Free Radic. Biol. Med. 43 (2007) 1639–1648.

[20] S.J. Yang, H.B. IglayReger, H.C. Kadouh, P.F. Bodary, Inhibition of the chemokine (C–C motif) ligand 2/chemokine (C–C motif) receptor 2 pathway attenuates hyperglycaemia and inflammation in a mouse model of hepatic steatosis and lipoatrophy, Diabetologia 52 (2009) 972–981.

[21] S. Lally, C.Y. Tan, D. Owens, G.H. Tomkin, Messenger RNA levels of genes involved in dysregulation of postprandial lipoproteins in type 2 diabetes: the role of Niemann-Pick C1-like 1, ATP-binding cassette, transporters G5 and G8, and of microsomal triglyceride transfer protein, Diabetologia 49 (2006) 1008–1016.

[22] P.P. Simonen, H.K. Gylling, T.A. Miettinen, Diabetes contributes to cholesterol metabolism regardless of obesity, Diabetes Care 25 (2002) 1511–1515.