Biochemical and Biophysical Research Communications 464 (2015) 674–677

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Ingestion of eicosapentaenoic acid in the early stage of social isolation reduces a fibroblast growth factor 21 resistant state independently of body weight in KKA^{y} mice

Katsunori Nonogaki*, Tomoe Yamazaki, Mari Murakami, Takao Kaji

Department of Diabetes Technology, Tohoku University, Graduate School of Biomedical Engineering, 6-6-11 Aramakiaza-Aoba, Aoba-ku, Sendai, Miyagi, 980-8579, Japan

ARTICLE INFO

Article history:
Received 2 July 2015
Accepted 11 July 2015
Available online 15 July 2015

Keywords:
EPA
FGF21
GLP-1
Hyperglycemia
Hypertriglyceridemia
ChREBP
SREBP1c
Periostin
gp78
Notch
Lipogenesis

ABSTRACT

Fibroblast growth factor (FGF) 21 is a mediator of glucose and lipid metabolism. Although exogenous administration of FGF21 exerts beneficial effects on glucose and lipid metabolism, circulating FGF21 levels are elevated in ob/ob and db/db mice, diet-induced obese mice and obese human. Here we show that ingestion of eicosapentaenoic acid (EPA) for 6 days after individually-housing significantly suppressed the hyperglycemia and hypertriglyceridemia associated with decreases in plasma insulin and FGF21 levels in KKA^{y} mice while having no effects on food intake, body weight or plasma active GLP-1 levels. The ingestion of EPA had no significant effects on the expression of FGF21 in the liver, epididymal white adipose tissue and skeletal muscle. Moreover, the ingestion of EPA significantly decreased the expression of hepatic peroxisome sterol regulatory element-binding protein (SREBP1c), carbohydrate response element–binding protein (ChREBP), stearoyl-CoA deaturase and periostin, which are involved in hepatic lipogenesis and hepatosteaotosis, in KKA^{y} mice. On the other hand, the ingestion of EPA had no significant effects on expression of hepatic gp78, Notch, forkhead box protein O1 or glucose-6-phosphatase. These findings suggest that EPA ingestion in the early stage of social isolation suppresses hyperglycemia and hypertriglyceridemia associated with reduced FGF21 and insulin resistance without altering food intake and body weight, and that the EPA ingestion suppresses hepatic lipogenesis by suppressing Notch- and gp78-independent SEREBP1c and ChREBP pathways in KKA^{y} mice.

1. Introduction

Fibroblast growth factor (FGF) 21 is produced by the liver, adipose tissues, and skeletal muscle [1]. Administration of FGF21 reduces hepatic glucose production and plasma glucose levels while increasing insulin sensitivity and adipose tissue glucose uptake [1]. Moreover, FGF21 reduces hepatic and plasma triglyceride and body weight while activating brown adipose tissue [1].

In the rodent diet-induced obesity (DIO) [2], genetically obese db/db [3] and ob/ob mice [4], the expression of FGF21 in white adipose tissue and liver is increased. In humans, circulating FGF21 levels are increased in obesity [3] and patients with type 2 diabetes [5,6]. Moreover, DIO mice have increases in endogenous levels of FGF21 and respond poorly to exogenous FGF21 [7]. These findings suggest that obesity is an FGF21-resistant state.

Eicosapentaenoic acid (EPA) reportedly reduces hepatic *de novo* lipogenesis by suppressing the expression of hepatic sterol regulatory element-binding protein (SREBP1c) and stearoyl-CoA deaturase (SCD-1) in ob/ob mice [8] and diet-induced obese mice [9]. We previously reported that social isolation promotes obesity and hyperglycemia in KKA^{y} mice with ectopic overexpression of agouti peptide, an endogenous melanocortin-4 receptor antagonist [10]. The effects of EPA on FGF21 resistant state and metabolic abnormalities induced by social isolation in KKA^{y} mice, however, have not been evaluated.

To determine the effects of EPA ingestion on the metabolic abnormalities in the early stage of social isolation, we examined the effects of ingestion of EPA for 6 days on body weight, food intake, blood glucose, plasma triglyceride, insulin, active GLP-1 and FGF21 levels, the expression of FGF21 in the liver, epididymal white adipose tissue (eWAT) and skeletal muscle, and genes involved in the

* Corresponding author.
E-mail addresses: katsu@trc.med.tohoku.ac.jp, knonogaki-tky@umin.ac.jp (K. Nonogaki).

http://dx.doi.org/10.1016/j.bbrc.2015.07.058
0006-291X/

regulation of hepatic lipogenesis and hepatic glucose production in individually-housed KKA^{y} mice.

2. Materials and methods

2.1. General procedures

KKA^{y} mice (4-wk-old males) were purchased from Japan CLEA (Tokyo). All mice were group-housed and acclimated to the colony for 1 wk before beginning the experiment. All mice were housed in cages with free access to water and a fish meal-free diet (fish meal-free F1: 4.4% fat; Funabashi Farm, Funabashi, Japan) on a 12-h light–dark cycle (lights off 20:00 h) in a temperature-controlled (20–22 °C) environment.

1 wk later, the mice were housed in individual cages with free access to water and a fish meal-free diet (fish meal-free F1: 4.4% fat; Funabashi Farm, Funabashi, Japan) or EPA diet (the fish meal-free diet plus EPA ethyl ester [5%]) over 6 days. The doses of EPA were selected as described previously [9]. At the end of the 6 days, the animals were decapitated, and blood was collected for measurements of blood glucose and plasma triglyceride levels. The liver, eWAT and soleus muscle were excluded for determining mRNA levels.

Mean food consumption per week was evaluated in 5 to 6-wk-old animals. Body weight was measured at 5 wk and 6 wk (before and after the 6 day feeding period). The experiment was performed between 10:00–12:00.

2.2. Blood chemistry

Blood glucose levels were measured using glucose strips (Blood glucose monitoring system; FreeStyle, NIPRO, Tokyo, Japan). Whole blood was mixed with EDTA-2Na (2 mg/mL) and aprotinin (500 kIU/mL) to determine the plasma triglyceride and insulin levels. Plasma triglyceride levels were measured by the GPO-HDAOS glycerol blanking method using L-type triglyceride H (Wako Pure Chemical Industries, Ltd, Japan). Plasma insulin levels were measured by radioimmunoassay (rat insulin RIA kit; Millipore Corporation, Billerica, MA, USA). The plasma levels of FGF21 were measured by ELISA (rat/mouse FGF21 ELISA kits; R&D system, Tokyo, Japan). Plasma levels of active GLP-1 were measured by an enzyme-linked immunosorbent assay (mouse active GLP-1 ELISA kit; Shibayagi Inc., Gunma, Japan) as described previously [11]. EPA was a kind gift from Mochida Pharmaceutical Co., Japan.

The animal studies were conducted in accordance with the institutional guidelines for animal experiments at the Tohoku University Graduate School of Medicine.

2.3. Real-time quantitative RT-PCR

Total RNA was isolated from mouse liver and soleus muscle using the RNeasy Midi kit (Qiagen, Hilden, Germany) and eWAT using the RNeasy Lipid Tissue Midi kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. cDNA synthesis was performed using a Super Script III First-Strand Synthesis System for RT-PCR Kit (Invitrogen, Rockville, MD) with 1 μg total RNA. cDNA synthesized from total RNA was evaluated in a real-time PCR quantitative system (LightCycler Nano Instrument Roche Diagnostics, Mannheim, Germany). The primers used are listed in Table 1. The relative amount of mRNA was calculated using β-actin mRNA as the invariant control. Data are shown as fold-change of the mean value of the control group, which received saline as described previously [10].

Table 1
Primers used for real-time RT-PCR.

Gene	Primer	
Notch	Sense	ATGTGGATGCTGCTGTTGTGCTCC
	Antisense	CCGGTTGGCAAAGTGGTCCA
G6Pase	Sense	TGCAAGGGAGAACTCAGCAA
	Antisense	GGACCAAGGAAGCCACAATG
FOXO1	Sense	GCGTGCCCTACTTCAAGGATAA
	Antisense	TCCAGTTCCTTCATTCTGCACT
SREBP1c	Sense	GCCGTGGTGAGAAGCGCACAGCCC
	Antisense	CAAGACAGCAGATTTATTCAGCTTTGC
ChREBP	Sense	CAGGGAATACACGCCTACAG
	Antisense	CAGGTGGGATCTTGGTCTTA
SCD-1	Sense	CCTTTCTGTCCATCCTCTGAAC
	Antisense	TAGGCACATTGAACACGAAGAC
gp78	Sense	CCTGTCCCTGCTGATTGCTA
	Antisense	CTCACGGTCACAAGAAGAGACT
Periostin	Sense	CCTCTATCCAGCAGATATTCCA
	Antisense	CTGCCACGAACAAACTTGA
FGF21	Sense	CACCGCAGTCCAGAAAGTC
	Antisense	ATCAAAGTGAGGCGATCCA

3. Statistical methods

Data are presented as mean ± SEM (n = 6). Comparisons between two groups were performed using Student's *t* test. A P value of less than 0.05 was considered statistically significant.

4. Results

4.1. Effects of EPA on food intake, body weight, blood glucose and plasma triglyceride, insulin and active GLP-1 levels in individually-housed KKA^{y} mice

Although the ingestion of a fish meal-free diet with EPA over 6 days had no effect on food intake (Fig. 1 A), body weight (Fig. 1B) or plasma active GLP-1 levels (Fig. 1F), the EPA treatment significantly decreased blood glucose (Fig. 1C), plasma triglyceride (Fig. 1D), insulin (Fig. 1E) levels compared with controls in individually-housed KKA^{y} mice compared with controls.

4.2. Effects of EPA on plasma FGF21 and the gene expression of FGF21 in the liver, eWAT and soleus muscle in individually-housed KKA^{y} mice

The ingestion of a fish meal-free diet with EPA over 6 days significantly increased plasma FGF21 levels (Fig. 2A), although there were no significant differences in FGF21 mRNA levels of the liver, eWAT and soleus muscle between controls without EPA and group treated with the EPA (Fig. 2B). These findings suggest that the decreases in plasma FGF21 levels are not due to the decreased expression of FGF21 in the peripheral tissues.

4.3. Effects of EPA on the gene expression involved in the regulation of hepatic lipogenesis and glucose production in individually-housed KKA^{y} mice

Ingestion of a fish meal-free diet with EPA over 6 days significantly decreased the expression of hepatic carbohydrate response element–binding protein (ChREBP), SREBP1c, SCD1 and periostin mRNA levels which are involved in hepatic lipogenesis and hepatosteaotosis while having no significant effect on gp78 (Fig. 3A). On the other hand, the EPA treatment had no significant effects on the expression of hepatic Notch, Forkhead box O1 (FOXO1) and

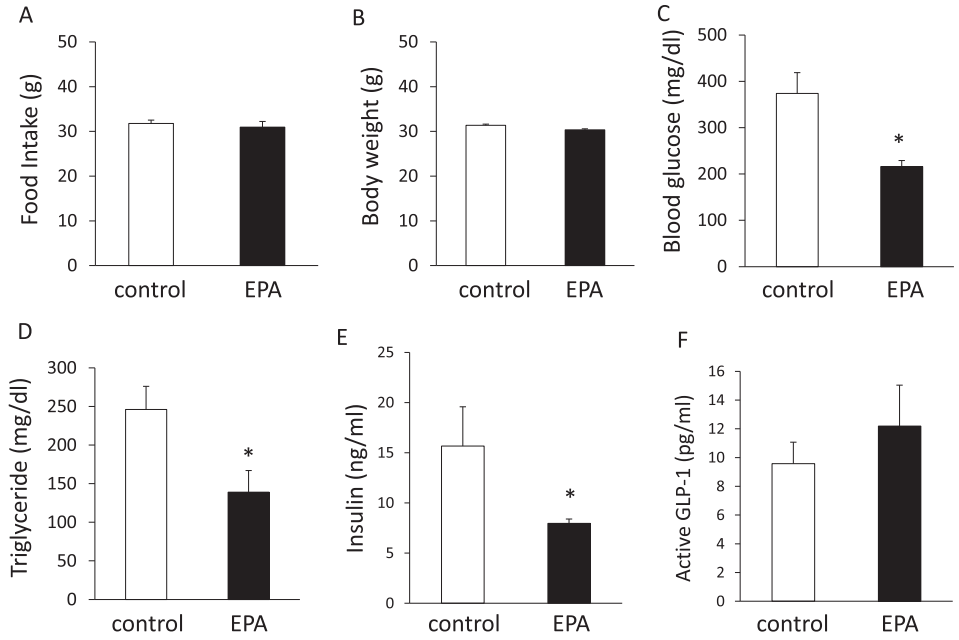

Fig. 1. Effects of ingestion of a fish meal-free diet with or without EPA (5%) over 6 days on food intake (A), body weight (B), blood glucose (C) and plasma triglyceride (D), insulin (E) and active GLP-1 levels (F) in individually-housed KKA^{y} mice. Basal body weight in 5-wk-old KKA^{y} mice treated with or without EPA was 25.1 ± 0.4 g and 25.2 ± 0.5 g, respectively. Data are presented as the mean values ± SEM (n = 6 for each group of animals). *P < 0.05.

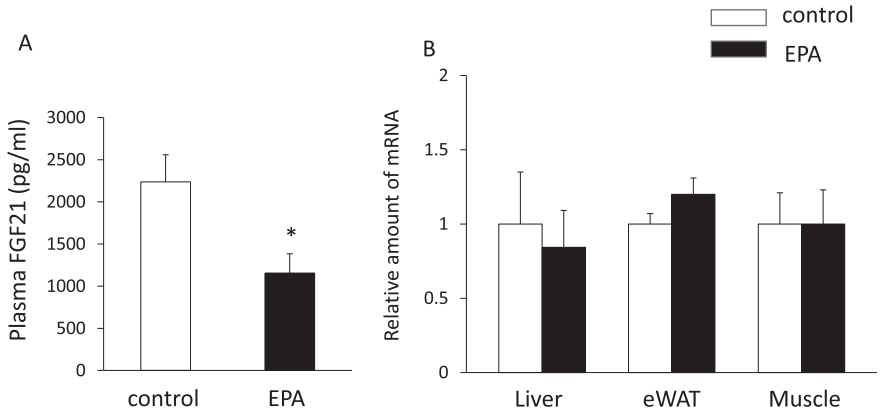

Fig. 2. Effects of ingestion of a fish meal-free diet with or without EPA (5%) over 6 days on plasma FGF21 levels (A) and the expression of FGF21 in the liver, eWAT and soleus muscle (B) in individually-housed KKA^{y} mice. Data are presented as the mean values ± SEM (n = 6 for each group of animals). *P < 0.05.

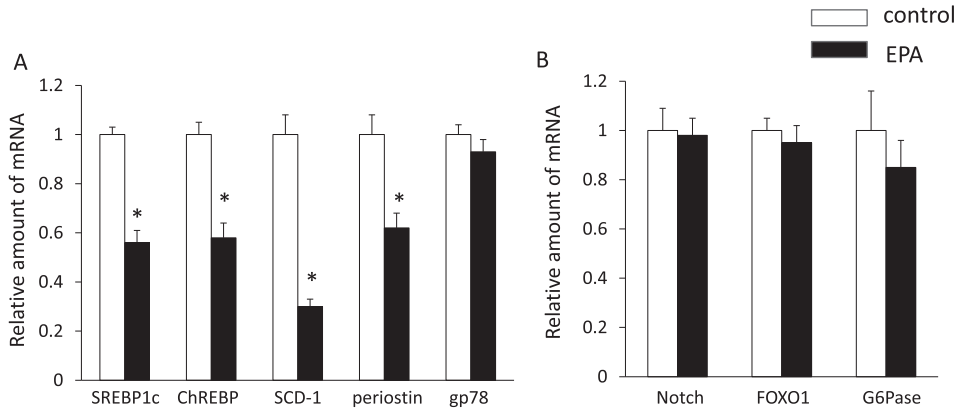

Fig. 3. Effects of ingestion of a fish meal-free diet with or without EPA (5%) over 6 days on the expression of hepatic genes involved in the regulation of triglyceride (A) and glucose (B) metabolism, in individually-housed KKA^{y} mice. Data are presented as the mean values ± SEM (n = 6 for each group of animals). *P < 0.05.

glucose-6-phosphatase (G6Pase), which are involved in hepatic glucose production (Fig. 3B).

5. Discussion

The results of the present study demonstrated that the EPA ingestion in the early stage of social isolation reduced hyperglycemia and hypertriglyceridemia associated with decreases in plasma insulin and FGF21 levels while having no effects on food intake and body weight. Because the decreases in plasma FGF21 levels induced by the EPA ingestion are not due to the decreased FGF21 production in the peripheral tissues including liver, eWAT and skeletal muscle, the decreases in plasma FGF21 induced by the EPA ingestion might be due to a reduced FGF21 resistant state. Moreover, the reduced FGF21 resistant state induced by the EPA ingestion might be related to the improvement of hyperglycemia, hyperinsulinemia and hypertriglyceridemia rather than obesity in KKA^y mice.

Because the EPA ingestion had no effects on the expression of hepatic Notch, FoxO1 and G6Pase, the suppressive effects of EPA on hyperglycemia in KKA^y mice are due to the suppression of Notch-mediated hepatic glucose production. The results of the present study demonstrated that the EPA ingestion reduced hyperinsulinemia without altering active GLP-1 levels in individually-housed KKA^y mice. EPA reportedly reduces insulin resistance by suppressing inflammatory cytokines such as tumor necrosis factor-α and interleukin-6 [12–14], and by increasing plasma adiponectin in obese rodents and humans [15]. The suppressive effects of the EPA ingestion on hyperglycemia in KKA^y mice might be due to the reduced insulin and FGF21 resistant state.

Insulin promotes hepatic fatty acid synthesis and the accumulation of triglycerides by SREBP1c induction [16]. Our results of the present study demonstrated that the EPA ingestion suppressed the insulin-induced SREBP1c pathway of hepatic lipogenesis in individually-housed KKA^y mice. In the liver, Notch signaling and gp78, a membrane-anchored ubiquitin ligase, reportedly upregulate SREBP1c-mediated lipogenesis [17,18]. However, the Notch and gp78 unlikely contributed to the suppressive effects of EPA on the expression of hepatic SREBP1c in KKA^y mice.

On the other hand, glucose promotes hepatic fatty acid synthesis and the accumulation of triglycerides by ChREBP induction [16]. The glucose-induced ChREBP induces the expression of hepatic periostin, which is a critical contributing factor in the regulation of hepatic triglyceride homeostasis and the development of obesity-induced hepatosteatosis [19]. Our results demonstrated that the EPA ingestion suppressed the glucose-induced ChREBP and periostin pathway in individually-housed KKA^y mice. In summary, these findings suggest that the EPA ingestion in the early stage of social isolation suppresses hyperglycemia and the FGF21 resistant state without altering food intake and body weight, and that the EPA ingestion suppresses hypertriglyceridemia by suppressing Notch- and gp78-independent hepatic SEREBP1c and ChREBP pathways of lipogenesis in KKA^y mice.

Conflict of interests

The authors declare no conflict of interests.

Acknowledgments

We are indebted to N. Satoh and M. Hazama for their skillful technical assistance. This work was supported by a Grant in-Aid for Scientific Research.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.07.058.

References

[1] M.L. Reitman, FGF21 mimetic shows therapeutic promise, Cell Metab. 18 (2013) 307–309.
[2] E.S. Muise, B. Azzolina, D.W. Kuo, M. EL-Sherbeini, Y. Tan, X. Yuan, J. Mu, J.R. Thompson, J.P. Berger, K.K. Wong, Adipose tissue fibroblast growth factor 21 is up-regulated by peroxisome proliferator-activated receptor gamma and altered metabolic states, Mol. Pharmacol. 74 (2008) 403–412.
[3] X. Zhang, D.C. Yeung, M. Karpisek, D. Stejskal, Z.G. Zhou, F. Liu, R.L. Wong, W.S. Chow, A.W. Tso, K.S. Lam, A. Xu, Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans, Diabetes 57 (2008) 1246–1253.
[4] M.K. Badman, A.R. Kennedy, A.C. Adams, P. Pissios, E. Maratos-Flier, A very low carbohydrate ketogenic diet improves glucose tolerance in ob/ob mice independently of weight loss, Am. J. Physiol. Endocrinol. Metab. 297 (2009) E1197–E1204.
[5] L. Li, G. Yang, H. Ning, M. Yang, H. Liu, W. Chen, Plasma FGF-21 levels in type 2 diabetic patients with ketosis, Diabetes Res. Clin. Pract. 82 (2008) 209–213.
[6] K. Li, L. Li, M. Yang, H. Zong, H. Liu, G. Yang, Effects of rosiglitazone on fasting plasma fibroblast growth factor 21 levels I patients with type 2 diabetes mellitus, Eur. J. Endocrinol. 161 (2009) 391–395.
[7] F.M. Fisher, P.C. Chui, P.J. Antonellis, H.A. Bina, A. Kharitonenkov, J.S. Flier, E. Maratos-Flier, Obesity is a fibroblast growth factor 21 (FGF21)-resistant state, Diabetes 59 (2010) 2781–2789.
[8] M. Sekiya, N. Yahagi, T. Matsuzaka, Y. Najima, M. Nakakuki, R. Nagai, S. Ishibashi, J. Osuga, N. Yamada, H. Shimano, Polyunsaturated fatty acids ameliorate hepatic steatosis in obese mice by SREBP-1 suppression, Hepatology 38 (2003) 1529–1539.
[9] A. Sato, H. Kawano, T. Notsu, M. Ohta, M. Nakakuki, K. Mizuguchi, M. Itoh, T. Suganami, Y. Ogawa, Antiobesity effect of eicosapentaenoic acid in high-fat/high-sucrose diet-induced obesity: importance of hepatic lipogenesis, Diabetes 59 (2010) 2495–2504.
[10] K. Nonogaki, K. Nozue, Y. Oka, Social isolation affects the development of obesity and type 2 diabetes in mice, Endocrinology 148 (2007) 4658–4666.
[11] K. Nonogaki, M. Hazama, N. Satoh, Liraglutide suppresses obesity and hyperglycemia associated with increases in hepatic fibroblast growth factor 21 production in KKA^y mice, Biomed. Res. Int. 2014 (2014) 751930.
[12] T. Shimizu, T. Iwamoto, S. Itou, N. Iwata, T. Endo, M. Takasaki, Effect of ethyl icosapentaenoate (EPA) on the concentration of tumor necrosis factor (TNF) and interleukin-1 (IL-1) in the carotid artery of cuff-sheathed rabbit models, J. Atheroscler. Thromb. 8 (2001) 45–49.
[13] H.W. Hsueh, Z. Zhou, J. Whelan, K.G. Allen, N. Moustaid-Moussa, H. Kim, K.J. Claycombe, Stearidonic and eicosapentaenoic acids inhibit interleukin-6 expression in ob/ob mouse adipose stem cells via Toll-like receptor-2-mediated pathways, J. Nutr. 141 (2011) 1260–1266.
[14] M. Figueras, M. Olivan, S. Busquets, F.J. López-Soriano, J.M. Argilés, Effects of eicosapentaenoic acid (EPA) treatment on insulin sensitivity in an animal model of diabetes: improvement of the inflammatory status, Obesity 19 (2011) 362–369.
[15] M. Itoh, T. Suganami, N. Satoh, K. Tanimoto-Koyama, X. Yuan, M. Tanaka, H. Kawano, T. Yano, S. Aoe, M. Takeya, A. Shimatsu, H. Kuzuya, Y. Kamei, Y. Ogawa, Increased adiponectin secretion by highly purified eicosapentaenoic acid in rodent models of obesity and human obese subjects, Arterioscler. Thromb. Vasc. Biol. 27 (2007) 1918–1925.
[16] L.L. Stein, M.H. Dong, R. Loomba, Insulin sensitizers in nonalcoholic fatty liver disease and steatohepatitis: current status, Adv. Ther. 26 (2009) 893–907.
[17] P. Bi, T. Shan, W. Liu, F. Yue, X. Yang, X.R. Liang, J. Wang, J. Li, N. Carlesso, X. Liu, S. Kuang, Inhibition of notch signaling promotes browning of white adipose tissue and ameliorates obesity, Nat. Med. 20 (2014) 911–918.
[18] T.F. Liu, J.J. Tang, P.S. Li, Y. Shen, J.G. Li, H.H. Miao, B.L. Li, B.L. Song, Ablation of gp78 in liver improves hyperlipidemia and insulin resistance by inhibiting SREBP to decrease lipid biosynthesis, Cell Metab. 16 (2012) 213–225.
[19] T. Wu, S. Wu, G. Ouyang, Periostin: a new extracellular regulator of obesity-induced hepatosteatosis, Cell Metab. 20 (2014) 562–563.