Fluoxetine Induces Hepatic Lipid Accumulation Via Both Promotion of the SREBP1c-Related Lipogenesis and Reduction of Lipolysis in Primary Mouse Hepatocytes

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Keywords

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SUMMARY

Aims: In this study, we investigated the peripheral mechanisms underlying the metabolic side effects of fluoxetine (FLX) by focusing on hepatic lipid metabolism. **Methods:** Primary mouse hepatocytes were prepared from male mice by the two-step perfusion method. The lipid accumulation in primary mouse hepatocytes was analyzed via neutral oil staining. And the lipid metabolism enzymes were determined with RT-PCR and Western blot. **Results:** Fluoxetine significantly induced the lipid accumulation in primary mouse hepatocytes. Moreover, FLX increased the acetyl-CoA carboxylase 1 (ACC1) and fatty acid synthase (FAS) expression, which are important enzymes in lipogenesis. Oppositely, Fluoxetine significantly decreased the carboxylesterase 3 (CES3) and carboxylesterase 1 (CES1) expression, which are related to lipolysis. Further study demonstrated FLX-activated SREBP1c, which is one of the most important transcription factors conducting coordinated transcriptional regulation of lipogenesis gene such as ACC1 and FAS. And the increase of lipogenesis gene (ACC1) was abolished by SB203580 but not by pyrrolidine dithiocarbamate (PDTC), suggesting through p38-MAPK pathway. **Conclusion:** Fluoxetine induces hepatic lipid accumulation via both promotion of the SREBP1c-related lipogenesis and reduction of lipolysis in primary mouse hepatocytes.

Introduction

Major depressive disorder (MDD) is a serious public health problem, which is associated with significant morbidity, mortality, disability, and suffering [1–3], and will be the second greatest contributor to the global burden of disease by 2020 [4]. Selective serotonin reuptake inhibitors (SSRIs) are the most common drugs for MDD [5,6]. As one of the most wide acceptant antidepressants [6–8], fluoxetine (FLX) is reported to have high possibility of weight gain and dyslipidemia, and more importantly, increasing the risk of obesity-related metabolic diseases such as diabetes mellitus, hypertension, and coronary heart diseases [9–11].

Mechanisms of weight gain and dyslipidemia resulting from FLX treatment are still poorly understood. The causes of SSRI-induced side effects could be multifactorial, involving actions on multiple serotonin receptors and a number of additional systems including histamine-1, opiate receptors in appetite regulation and carbohydrate intake, demonstrated by studies working on central nervous system [12,13]. However, further insight into their mechanisms of actions is still warranted. By now, few studies examined

the direct effect of antidepressants on peripheral lipid metabolic organs and tissues [14].

The liver is an organ of major importance in lipid metabolism. Among the important lipogenesis genes, fatty acid synthase (FAS) is the key synthetic enzyme of triglyceride (TG) [15]. Similarly, acetyl-CoA carboxylase (ACC), which synthesizes malonyl-CoA from acetyl-CoA, is also of particular importance in TG biosynthesis. ACC1 is predominantly expressed in lipogenic tissues such as liver and adipose [16]. In the liver, it has been revealed that SREBP1 is one of the most important transcription factors conducting coordinated transcriptional regulation of lipogenesis genes [17]. SREBP1c, one of the two splice variants of SREBP1, is by far the most abundant isoform in the liver as a key transcription factor for the regulation of lipogenic gene expression and by extension lipid storage in liver [18].

On the contrary, lipolysis genes are also indispensable to participate in lipid metabolism. Although the importance of hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) in the adipose tissue has been clearly defined [19], the role of these lipases in hepatic TG metabolism is less clear due to their very low expression levels in liver [20]. Carboxylester-

ases, endoplasmic reticulum-localized TG hydrolases, belong to a, b -serine hydrolase multigene family and are widely expressed in multiple mammalian tissues [21]. There are abundant carboxylesterases in the liver [22]. Carboxylesterase 3 (CES3, also called CES1d) in mouse, correspondingly carboxylesterase1 (CES1) in human, has been suggested to be one of hepatic TG hydrolases involved in hepatic TG turnover [23]. Mouse CES1 is a homolog of CES3, possessing a conserved esterase/lipase active site motif, which suggests that it plays a role in hepatic TG metabolism as human CES1 does [24].

In this study, we demonstrated that FLX-induced hepatic lipid accumulation in the primary mouse hepatocytes. On the basis of the information mentioned above, we hypothesize that FLX is likely to affect hepatic lipid metabolism by promoting the SREBP1c-related lipogenesis and reducing lipolysis in the primary mouse hepatocytes. This study will help to further supplement peripheral mechanisms of metabolic side effects resulting from FLX and other antidepressants.

Materials and Methods

Animals and Reagents

Male ICR mice, 18–22 g, were obtained from the Experimental Animal Center of Nanjing (Nanjing, China). They were kept in a temperature-controlled environment ($22 \pm 2^\circ\text{C}$), with $55 \pm 5\%$ relative humidity and a 12-h light–dark cycle, and fed with standard chow, for at least 1 week before any experiments. The use of animals was approved by IACUC (Institutional Animal Care and Use Committee) of Nanjing Medical University. Every effort was made to minimize animal suffering and to reduce the number of animals used for experiments.

Fluoxetine and Oil Red O were from Sigma (St Louis, MO, USA). Williams' Medium E (WME), TRIzol, and high-fidelity platinum Taq DNA polymerase were produced by Invitrogen (Carlsbad, CA, USA). MLV reverse transcriptase and RNase inhibitor by Promega (Madison, WI, USA). Antibodies used in this study were as follows: Anti-ACC1 (Abcam, Cambridge, UK), anti-hCES1 and anti-hCES2 (Abgent, San Diego, CA, USA), anti-SREBP1c (Anbo, Changzhou, China), anti-FAS, and anti- β -actin (Bioworld, St. Louis Park, MN, USA). All other reagents were of analytical grade and commercially available.

Primary Mouse Hepatocytes Culture

Primary mouse hepatocytes were prepared from male ICR mice referred to a modification of the two-step perfusion method as described previously [25]. Briefly, fasted mice (12 h) were anesthetized by i.p. injected urethane (1 g/kg body weight). When mice were completely anesthetized, surgery was performed to expose the liver. Livers were perfused *in situ* through the portal vein with calcium-free buffer at pH 7.4. A second perfusion was performed containing 100 mM HEPES buffer at pH 7.6, with an addition of 0.5 mg/mL collagenase IV (Sigma-Aldrich, St. Louis, MO, USA) at a flow rate of 0.7 mL/min for about 10 min. All solutions were prewarmed in a 40°C water bath. The liver was then transferred to a sterile dish containing wash medium supplemented with 1% penicillin–streptomycin and swung to be

dispersed. Primary mouse hepatocytes were washed twice with wash medium by two successive centrifugation steps of $50 \times g$ for 2 min at 4°C . After the last wash, primary mouse hepatocytes were suspended in the WME supplemented with 10% FBS. At this step, cell viability, assessed by trypan blue dye exclusion, was typically 85–95%. Primary mouse hepatocytes were plated at 2×10^6 cells per collagen-coated six-well plate in 5% FBS Williams E medium (WME) containing insulin–transferrin–selenium supplement and 100 U/mL penicillin/10 $\mu\text{g/mL}$ streptomycin, dexamethasone (0.1 μM), and were maintained at 37°C , in a humidified atmosphere of 5% CO_2 for 4 h to allow attachment. Primary mouse hepatocytes were then washed with PBS and supplied with fetal bovine serum-free medium. After being continued cultured for 3 days with a change of fresh medium at 36 h, primary mouse hepatocytes were treated with FLX (2.5, 10, 25, and 50 μM) or 0.1% DMSO for 24 h.

Neutral Oil Staining

Primary mouse hepatocytes were treated with FLX (2.5, 10, and 25 μM) or 0.1% DMSO for 24 h. Primary mouse hepatocytes were washed extensively D-Hanks solution and fixed with 4% paraformaldehyde for 20 min at room temperature. The fixed cells were stained with freshly diluted Oil Red O solution (0.5% Oil Red O in isopropanol: $\text{H}_2\text{O} = 3:2$) for 1 h at 37°C and rinsed with D-Hanks solution. The primary mouse hepatocytes were visualized for microphotographs microscopically using a Leica DM6000B digital light microscope (Leica Microsystems, Wetzlar, Germany).

Reverse Transcription-Polymerase Chain Reaction

Total RNA was isolated by using TRIzol and checked by 1.5% agarose gel electrophoresis for quality control. The first-strand cDNA was synthesized using total RNA (1 μg) at 25°C for 10 min, 42°C for 50 min, and 70°C for 10 min using oligo(dT) and M-MLV reverse transcriptase. The primers used for mouse CES1 (NM_145603.2), CES3 (NM_053200.2) and GAPDH were as follows: CES1 forward, TTCAAGGATGTCAGACCACC and reverse, AACACATTTTTTTTGATACAGGGTA; CES3 (CES1d) forward, GGCATCAACAAGCAAGAGTTTGGC and reverse, CTTTTTGGTG AGGTGATCTGTCCC; GAPDH forward, GTATGTC GTGGAGTCT ACTGGTGTC and reverse, GGTGCAGGATGCATTGCTGACATTC. The thermal cycling conditions of the PCR were 94°C for 5 min, followed by 23–35 cycles for 20 s at 94°C , 20 s at 64°C , 1 min at 72°C , and a final extension at 72°C for 7 min. The amplified products were separated by electrophoresis in $0.5 \times$ Tris–acetate EDTA buffer with a 1.5% agarose gel containing 0.1 $\mu\text{g/mL}$ of ethidium bromide (6 μL). The DNA bands were visualized and analyzed by Biosens sc 810 Gel Electrophoresis Image analytic system (Shanghai, China). The ratios of CES1, CES3 mRNA, and GAPDH mRNA were calculated.

Western Blot Analysis

Primary mouse hepatocytes lysates (20 μg) were resolved by 7.5% SDS-polyacrylamide gel electrophoresis and electrophoretically

transferred to a nitrocellulose membrane. After nonspecific binding sites being blocked with 5% nonfat milk, the blots were incubated with an antibody against hCES1 (1:2500), hCES2 (1:2500), ACC1 (1:2500), FAS (1:1000), SREBP1c (1:1000), and β -actin (1:5000).

Statistical Analysis

Data were expressed as means $\pm$ SD. Statistical analysis for multiple comparisons was performed using SPSS software (SPSS, version 19, Chicago, IL, USA). The significance was made according to one-way ANOVA, followed by Dunnett's *post hoc* test. The statistical significance is defined as $P < 0.05$.

Results

FLX-Induced Lipid Accumulation in Primary Mouse Hepatocytes

Primary mouse hepatocytes were treated with different concentrations of FLX or the same volume of DMSO for 24 h. As shown in Figure 1, both the number and volume of lipid droplets in the cytoplasm were increased in a concentration-dependent manner after the treatment of FLX (2.5–25 μ M) for 24 h, implying FLX increased neutral lipid accumulated in the primary hepatocytes. Meanwhile, there was no significant difference of cell viability observed between the FLX-treated and control cells when the concentration of FLX was no more than 25 μ M, while some morphologic changes were observed at 25 μ M FLX such as nucleus presented small size and cluster together. But the cell viability was

significantly decreased, and the nucleus showed small size, meta-chromatin condensed, rows or cluster (presented apoptosis) by treatment of 50 μ M FLX (data not shown) for 24 h. Therefore, a maximal concentration of 25 μ M was used in the following experiments.

FLX upregulated the Lipogenesis-Related Genes ACC1 and FAS Expression via SREBP1c Activation

To investigate the mechanisms of the lipid accumulation induced by FLX, the expression of two key enzymes in the de novo hepatic fatty acid synthesis, ACC1 and FAS, was determined. Primary hepatocytes were exposed to FLX at 2.5, 10, and 25 μ M for 24 h. As shown in Figures 2A,B, the expression of ACC1 and FAS was repeatedly increased by FLX in a concentration-dependent manner. The data suggested that promoted lipid accumulation and TG production (data not shown) caused by FLX were related to the increased expression of ACC1 and FAS, two enzymes related to lipogenesis. SREBP1c, as an important transcriptional factor in regulating the expression of enzymes ACC and FAS, plays an essential role in hepatic TG synthesis, so we examined the expression of SREBP1c. As expected, SREBP1c expression significantly increased by FLX in primary mouse hepatocytes (Figure 3A). Furthermore, we investigated the truncated nuclear (active) form of SREBP1c (n-SREBP1c) expression. In result, FLX increased the n-SREBP1c expression (Figure 3B). The data suggested that FLX increased not only the precursor of SREBP1c (125 kDa) expression, but also the active form of n-SREBP1c (68 kDa). These findings indicated that the upregulation of ACC1 and FAS induced by FLX was likely to be mediated by SREBP1c.

Figure 1 Fluoxetine (FLX) induces lipid accumulation in the primary mouse hepatocytes. Primary mouse hepatocytes were exposed to different concentrations of FLX (2.5, 10, 25 μ M) for 24 h. Then, primary mouse hepatocytes were fixed and stained with Oil Red O to observe the accumulation of neutral lipids (red). Cell nuclei were stained with hematoxylin (blue). Images are shown at 400 $\times$ magnification. The data are representative of images obtained from at least three times.

FLX upregulated the Lipogenesis-Related Genes through p38-MAPK Pathway but not NF- κ B Pathway

To investigate the mechanisms of the increase of ACC1 expression induced by FLX, the AMPK activator, 5-Aminoimidazole-4-carboxamide ribonucleoside (AICAR, 50 μ M), and the differentiated inhibitors, including ammonium pyrrolidine dithiocarbamate (PDTC, 10 μ M) and 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1Himidazole (SB203580, 10 μ M), were used 30 min before being treated with 10 μ M FLX in primary mouse hepatocytes. As shown in Figure 4, SB203580 abolished the increase of ACC1 induced by FLX, but PDTC did not. The data suggested that FLX increased the ACC1 expression through p38-MAPK pathway but not NF- κ B pathway.

In addition, AICAR, a compound that activates AMPK, almost abolishes the increase of ACC1, suggesting that FLX-induced hepatic lipid accumulation is likely to be related to the repression of AMPK.

FLX downregulated Lipolysis Genes CES 1 and CES 3

We next examined whether FLX affects the lipolysis in primary mouse hepatocytes. Lipolysis leading to the breakdown of TG into fatty acids and glycerol requires activity of TG lipases. The HSL and ATGL in the adipose tissue are very important enzymes in

Figure 2 Fluoxetine (FLX) increases the expression of hepatic fatty acid synthesis genes **(A)** FLX increases acetyl-CoA carboxylase 1 (ACC1) expression. **(B)** FLX increases fatty acid synthase (FAS) expression. Primary mouse hepatocytes were exposed to different concentrations of FLX (2.5, 10, 25 μM) for 24 h. Primary mouse hepatocytes lysates (20 μg) were analyzed for ACC1 and FAS using Western blot. * $P < 0.05$, ** $P < 0.01$, versus control group. Data represent means $\pm$ SD. All experiments described in this figure were repeated at least three times.

Figure 3 Fluoxetine (FLX) increases SREBP1c expression and its activation **(A)** FLX increases precursor, SREBP1c expression, and densitometric analysis. **(B)** FLX increases both precursor and mature SREBP1c (N-SREBP1c) Primary mouse hepatocytes were exposed to different concentrations of FLX (2.5, 10, 25 μM) for 24 h. Primary mouse hepatocytes lysates were prepared and analyzed for precursor and mature SREBP1c using Western blot. * $P < 0.05$, ** $P < 0.01$ versus control group. Data represent means $\pm$ SD. All experiments described in this figure were repeated at least three times.

lipolysis, but they are very low expressed in hepatocytes [17,18]. Therefore, we examined the other abundant lipases like mouse CES1 and CES3 in primary mouse hepatocytes, which are involved in hepatic lipolysis and TG turnover.

As results, FLX significantly decreased the CES1 and CES3 expression at mRNA level in the dose-dependent manner (Figure 5A). Very similarly, FLX also markedly decreased the CES1 and CES3 expression at protein level in the concentration-dependent manner (Figure 5B). The data implied that downregu-

Figure 4 The pathway of increase of acetyl-CoA carboxylase 1 (ACC1) mediated by v fluoxetine (FLX). Primary mouse hepatocytes were administrated with 5-Aminoimidazole-4-carboxamide ribonucleoside (50 μM), pyrrolidine dithiocarbamate (10 μM), SB203580 (10 μM) 30 min before being treated with FLX (10 μM) or the same volume of DMSO for 24 h. Primary mouse hepatocytes lysates were prepared, and lysates (20 μg) were subjected to Western analyses with an antibody against ACC1 or β-actin. All the experiments were repeated at least three times.

lation of CES1 and CES3 could be another target involved in FLX-induced hepatic lipid accumulation.

Discussion

Fluoxetine (also known by the trade names Prozac) is a widely used antidepressant of the SSRI class [26]. Despite the availability of newer agents, FLX remains extremely popular up to now [27]. The results of a patient drug safety monitoring service named iGuard.org in 2009 showed that weight gain occurred in about 49% of patients out of approximately 700 patients who were taking SSRI antidepressants [27]. A significant increase in body weight puts the patient at risk for conditions including coronary heart disease, hypertension, and type 2 diabetes and, importantly, for noncompliance with the treatment [28,29].

Present studies focused much more on the central mechanisms of the weight gain side effect of antidepressants [30,31] than on

Figure 5 Fluoxetine (FLX) decreases mouse carboxylesterase 3 (CES3) and CES1 both at mRNA and protein levels. **(A)** FLX decreases mouse CES3 and CES1 at mRNA level. **(B)** FLX decreases mouse CES3 and CES1 at protein level. Primary mouse hepatocytes were exposed to different concentrations of FLX (2.5, 10, 25 μ M) for 24 h. Total RNA and cell lysates were prepared and analyzed for CES3 and CES1 with RT-PCR and Western blot. * P < 0.05 versus control group. Data represent means $\pm$ SD. All experiments described in this figure were repeated at least three times.

the peripheral mechanisms. In the present study, we focused on the effects of FLX on liver that is a major organ of lipid metabolism. We demonstrated that exposure to FLX significantly increased both the number and volume of lipid droplets with using Oil Red O stain that is used for staining of neutral TG and lipids in primary mouse hepatocytes. FLX mainly affected the TG metabolism in primary mouse hepatocytes, which is consistent with some other studies [32,33]. More importantly, FLX-induced hepatic lipid accumulation and the alteration of TG metabolism may be a key to the metabolic side effects of antipsychotic drugs. The TG metabolism is included two processes, lipogenesis and reduction of lipolysis. Next, we examined some lipogenesis-related enzymes and found FLX significantly increased the ACC1 and FAS expression (Figure 2A,B), which are key enzymes for the biosynthesis [34].

SREBP1c is a key regulator of fatty acid metabolism and plays a pivotal role in transcriptional regulation of a number of lipogenic genes that mediate lipid synthesis [35]. SREBP-1c protein is present in two forms: a precursor, membrane-bound form and a mature form resulting from the proteolytic cleavage of the precursor and the release of the active, N-terminal part of the protein. This mature form is translocated into the nucleus, where it binds to specific sequences in the promoters of lipogenic genes including ACC1 and FAS [36,37]. To elucidate the possible mechanisms of TG accumulation induced by FLX in primary mouse hepatocytes, we investigated the expression of SREBP1c and its active form n-SREBP1c after FLX treatment. The results showed that the treatment with increasing concentrations of FLX resulted in upregulated both precursor p-SREBP1c (125 kDa) and its mature n-SREBP1c (68 kDa) expression (Figure 3A,B). The findings suggest that FLX induces ACC1 and FAS expression through increasing

SREBP1c expression and activating the function of SREBP1c. Therefore, SREBP-activating effect is thought to be important to the metabolic side effects of antidepressants and antipsychotics [14,38,39].

To investigate the mechanism for the increase of ACC1, which is one of SREBP1c target genes, expression mediated by FLX, the different selected signal pathway protein inhibitors are used. The results show that SB203580, a p38MAPK inhibitor [40], almost abolishes the increase of ACC1 protein level mediated by FLX, but PDTC, a NF- κ B inhibitor [41], does not (Figure 4). The data suggest FLX upregulates ACC1 through the p38MAPK pathway but not through the NF- κ B pathway. In addition, AICAR, a compound that activates AMPK, almost abolishes the increase of ACC1, suggesting that repression of AMPK is involved in the hepatic lipid accumulation induced by FLX. AMPK is an enzyme that plays a role in cellular energy homeostasis. Repression of AMPK is inhibition of hepatic fatty acid oxidation and stimulation of triglyceride synthase and lipogenesis [42]. And detail mechanism of FLX affecting AMPK signal pathway will be further clarified.

Hepatic lipid accumulation induced by FLX suggests two possibilities: (1) FLX promotes lipogenesis and/or (2) FLX reduces lipolysis. We have provided evidence to support the first possibility. Next, we investigate whether FLX affected the lipolysis. Carboxylesterases are initially known to be important to metabolize xenobiotics, like some pesticides and drugs [22], whereas endobiotics, such as TG, cholesterol esters, and 2-arachidonoylglycerol, are also substrates for carboxylesterases [43]. It is now clearly apparent that carboxylesterases, playing a role in lipid metabolism, have been used as a therapeutic target in the treatment of metabolic disorders, such as diabetes and atherosclerosis [19,23]. For example, carboxylesterases control in part the lipolysis of TG in mouse

adipose tissues, thus, the dysregulation of carboxylesterases results in high levels of fatty acids in the circulation and lipotoxicity, a clinical manifestation of diabetes [19]. Due to antibody against human CES1 strongly cross-reacts with mouse CES1d (mouse CES3) and antibody against human CES2 strongly cross-reacts with mouse CES1e (mouse CES1) [44], we used antibody against human CES1 and CES2 to detect the expression of mouse carboxylesterases. The results showed that FLX significantly increased the expression of CES3 and CES1 both at mRNA and protein levels (Figure 5A,B), implying mouse carboxylesterases were involved in hepatic lipid accumulation induced by FLX in the primary mouse hepatocytes. These results were supported by Zhu's study [45]. It could be speculated that the decrease in carboxylesterases might reduce the lipolysis of TG, which leads to TG accumulation in primary mouse hepatocytes. The finding hinted another possible mechanism of TG accumulation induced by FLX is mediated by the decreased expression of carboxylesterases. In addition, some studies speculated that human CES1 has a SREBP1-binding site at its promoter [46], hinting that the regulation of carboxylesterases caused by FLX may also relates to transcriptional factor SREBP1, but clear evidence will be further studied.

In summary, our work points out several important conclusions. First, FLX induces hepatic lipid accumulation. Potentially, long-term use of FLX is likely to lead to weight gain. Second, both

promotion of SREBP1c-related lipogenesis and reduction of lipolysis are involved in the hepatic lipid accumulation induced by FLX in the primary mouse hepatocytes, which is through p38-MAPK pathway. Third, this study specifically reports that FLX induces inhibition of carboxylesterases, which may be link FLX to an increase of lipid accumulation. These findings support that FLX induces metabolic adverse effects at least in part through direct disturbances of lipid homeostasis in the liver, maybe in the adipose tissue. FLX-mediated perturbation of lipid homeostasis in the peripheral tissues represents an important molecular mechanism involved in antidepressant-associated metabolic adverse effects.

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Conflict of Interest

The authors declare no conflict of interest.

References

- Insel TR, Charney DS. Research on major depression: strategies and priorities. *JAMA* 2003;**289**:3167–3168.
- Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. *Lancet* 2006;**367**:1747–1757.
- Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med* 2006;**3**:e442.
- Freeborough A, Kimpson J. Discovering new genetic and psychosocial pathways in Major Depressive Disorder: the NewMood project. *Psychiatr Danub* 2011;**23**(Suppl 1): S138–S141.
- Asarnow JR, Emslie G, Clarke G, et al. Treatment of selective serotonin reuptake inhibitor-resistant depression in adolescents: predictors and moderators of treatment response. *J Am Acad Child Adolesc Psychiatry* 2009;**48**:330–339.
- Severus E, Schaaff N, Möller HJ. State of the art: treatment of bipolar disorders. *CNS Neurosci Ther* 2012;**18**:214–218.
- Wong DT, Perry KW, Bymaster FP. Case history: the discovery of fluoxetine hydrochloride (Prozac). *Nat Rev Drug Discov* 2005;**4**:764–774.
- Desmarais JE, Beauclair L, Margolese HC. Switching from brand-name to generic psychotropic medications: a literature review. *CNS Neurosci Ther* 2011;**17**:750–760.
- Mastrorandi C, Paz-Filho GJ, Valdez E, Maestre-Mesa J, Licinio J, Wong ML. Long-term body weight outcomes of antidepressant-environment interactions. *Mol Psychiatry* 2011;**16**:265–272.
- Jazayeri S, Tehrani-Doost M, Keshavarz SA, et al. Comparison of therapeutic effects of omega-3 fatty acid eicosapentaenoic acid and fluoxetine, separately and in combination, in major depressive disorder. *Aust N Z J Psychiatry* 2008;**42**:192–198.
- Masand PS, Gupta S. Long-term side effects of newer-generation antidepressants: SSRIs, venlafaxine, nefazodone, bupropion, and mirtazapine. *Ann Clin Psychiatry* 2002;**14**:175–182.
- Cascade E, Kalali AH, Kennedy SH. Real-world data on SSRI antidepressant side effects. *Psychiatry (Edmont)* 2009;**6**:16–18.
- Warden D, Trivedi MH, Wisniewski SR, et al. Early adverse events and attrition in selective serotonin reuptake inhibitor treatment: a suicide assessment methodology study report. *J Clin Psychopharmacol* 2010;**30**:259–266.
- Yang LH, Chen TM, Yu ST, Chen YH. Olanzapine induces SREBP-1-related adipogenesis in 3T3-L1 cells. *Pharmacol Res* 2007;**56**:202–208.
- Menendez JA, Vazquez-Martin A, Ortega FJ, Fernandez-Real JM. Fatty acid synthase: association with insulin resistance, type 2 diabetes, and cancer. *Clin Chem* 2009;**55**:425–438.
- Cheng D, Chu CH, Chen L, et al. Expression, purification, and characterization of human and rat acetyl coenzyme A carboxylase (ACC) isozymes. *Protein Expr Purif* 2007;**51**:11–21.
- Ferre P, Foufelle F. Hepatic steatosis: a role for de novo lipogenesis and the transcription factor SREBP-1c. *Diabetes Obes Metab* 2010;**12**(Suppl 2):83–92.
- Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 2002;**109**:1125–1131.
- Quiroga AD, Lehner R. Liver triacylglycerol lipases. *Biochim Biophys Acta* 2012;**1821**:762–769.
- Reid BN, Ables GP, Otlivanchik OA, et al. Hepatic overexpression of hormone-sensitive lipase and adipose triglyceride lipase promotes fatty acid oxidation, stimulates direct release of free fatty acids, and ameliorates steatosis. *J Biol Chem* 2008;**283**:13087–13099.
- Cyglar M, Schrag JD, Sussman JL, et al. Relationship between sequence conservation and three-dimensional structure in a large family of esterases, lipases, and related proteins. *Protein Sci* 1993;**2**:366–382.
- Mao Z, Li Y, Peng Y, et al. Lipopolysaccharide down-regulates carboxylesterases 1 and 2 and reduces hydrolysis activity in vitro and in vivo via p38MAPK-NF-kappaB pathway. *Toxicol Lett* 2011;**201**:213–220.
- Dolinsky VW, Gilham D, Alam M, Vance DE, Lehner R. Triacylglycerol hydrolase: role in intracellular lipid metabolism. *Cell Mol Life Sci* 2004;**61**:1633–1651.
- Ko KW, Erickson B, Lehner R. Es-x/Ces1 prevents triacylglycerol accumulation in McArdle-RH7777 hepatocytes. *Biochim Biophys Acta* 2009;**1791**:1133–1143.
- Severgnini M, Sherman J, Sehgal A, et al. A rapid two-step method for isolation of functional primary mouse hepatocytes: cell characterization and asialoglycoprotein receptor based assay development. *Cytotechnology* 2012;**64**:187–195.
- Wong DT, Horng JS, Bymaster FP, Hauser KL, Molloy BB. A selective inhibitor of serotonin uptake: Lilly 110140, 3-(p-trifluoromethylphenoxy)-N-methyl-3-phenylpropylamine. *Life Sci* 1974;**15**:471–479.
- Raeder MB, Ferno J, Glambek M, Stansberg C, Steen VM. Antidepressant drugs activate SREBP and up-regulate cholesterol and fatty acid biosynthesis in human glial cells. *Neurosci Lett* 2006;**395**:185–190.
- Olsson M, Marcus SC. National patterns in antidepressant medication treatment. *Arch Gen Psychiatry* 2009;**66**:848–856.
- Kivimäki M, Hamer M, Batty GD, et al. Antidepressant medication use, weight gain, and risk of type 2 diabetes: a population-based study. *Diabetes Care* 2010;**33**:2611–2616.
- Virk S, Schwartz TL, Jindal S, Nihalani N, Jones N. Psychiatric medication induced obesity: an aetiological review. *Obes Rev* 2004;**5**:167–170.
- Fride E, Bregman T, Kirkham TC. Endocannabinoids and food intake: newborn suckling and appetite regulation in adulthood. *Exp Biol Med (Maywood)* 2005;**230**:225–234.
- Turner EH, Rosenthal R. Efficacy of antidepressants. *BMJ* 2008;**336**:516–517.
- Turner EH, Matthews AM, Linardatos E, Tell RA, Rosenthal R. Selective publication of antidepressant trials and its influence on apparent efficacy. *N Engl J Med* 2008;**358**:252–260.

34. Tong L. Acetyl-coenzyme A carboxylase: crucial metabolic enzyme and attractive target for drug discovery. *Cell Mol Life Sci* 2005;**62**:1784–1803.
35. Sato R. SREBPs: protein interaction and SREBPs. *FEBS J* 2009;**276**:622–627.
36. Foufelle F, Ferre P. New perspectives in the regulation of hepatic glycolytic and lipogenic genes by insulin and glucose: a role for the transcription factor sterol regulatory element binding protein-1c. *Biochem J* 2002;**366**:377–391.
37. Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* 1997;**89**:331–340.
38. Laressergues E, Staels B, Valeille K, et al. Antipsychotic drug action on SREBPs-related lipogenesis and cholesterologenesis in primary rat hepatocytes. *Naunyn Schmiedebergs Arch Pharmacol* 2010;**381**: 427–439.
39. Ferno J, Vik-Mo AO, Jassim G, et al. Acute clozapine exposure in vivo induces lipid accumulation and marked sequential changes in the expression of SREBP, PPAR, and LXR target genes in rat liver. *Psychopharmacology* 2009;**203**:73–84.
40. Lin ML, Lu YC, Chung JG, et al. Down-regulation of MMP-2 through the p38 MAPK-NF-kappaB-dependent pathway by aloe-emodin leads to inhibition of nasopharyngeal carcinoma cell invasion. *Mol Carcinog* 2010;**49**:783–797.
41. Mazzone GL, Rigato I, Ostrow JD, Tiribelli C. Bilirubin effect on endothelial adhesion molecules expression is mediated by the NF-kappaB signaling pathway. *Biosci Trends* 2009;**3**:151–157.
42. Peterson JM, Aja S, Wei Z, Wong GW. CTRP1 protein enhances fatty acid oxidation via AMP-activated protein kinase (AMPK) activation and acetyl-CoA carboxylase (ACC) inhibition. *J Biol Chem* 2012;**287**:1576–1587.
43. Ross MK, Crow JA. Human carboxylesterases and their role in xenobiotic and endobiotic metabolism. *J Biochem Mol Toxicol* 2007;**21**:187–196.
44. Xiao D, Chen YT, Yang D, Yan B. Age-related inducibility of carboxylesterases by the antiepileptic agent phenobarbital and implications in drug metabolism and lipid accumulation. *Biochem Pharmacol* 2012;**84**:232–239.
45. Zhu HJ, Appel DI, Peterson YK, Wang ZC, Markowitz JS. Identification of selected therapeutic agents as inhibitors of carboxylesterase 1: potential sources of metabolic drug interactions. *Toxicology* 2010;**270**:59–65.
46. Abu-Elheiga L, Almaraz-Ortega DB, Baldini A, Wakil SJ. Human acetyl-CoA carboxylase 2. Molecular cloning, characterization, chromosomal mapping, and evidence for two isoforms. *J Biol Chem* 1997;**272**:10669–10677.