Caspian Journal of Neurological Sciences
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The Involvement of Nitric Oxide in Antidepressant-Like Effect of Metformin after Bile-Duct Ligation in NMRI Mice

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ARTICLE INFO

Article type:
Original Article

Article history:
Received: 12 July 2015
Accepted: 5 September 2015
Available online: 6 October 2015
CJNS 2015; 1 (3): 1-10

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ABSTRACT

Background: In some disorders such as diabetes mellitus patients can display depressive symptoms. Metformin is among the first-line treatments for management of the type 2 diabetes mellitus which may have some anti-depressant effect.
Objective: Current investigation was performed to examine the anti-depressant effects of metformin and the involvement of nitric oxide (NO) in this way, in an experimental animal model of cholestasis in NMRI (Naval Medical Research Institute) mice.
Materials and Methods: Bile duct ligated (BDL) and sham-operated mice were forced to swim separately and the effect of metformin on immobility time in the last 4 minutes of the 6 minutes test was assessed. To evaluate the probable participation of NO, N-nitro-L-arginine methyl ester (L-NAME) a non-specific NO synthase inhibitor and aminoguanidine, a specific iNO synthase inhibitor were injected acutely to metformin-treated BDL mice and then their immobility time was calculated in forced swimming test (FST).
Results: The immobility time significantly reduced after bile-duct ligation and metformin-treatment decreased this time additionally. L-NAME but not amino-guanidine administration significantly inhibited antidepressant like property of metformin in BDL mice. We have displayed that NO overproduction by metformin in cholestatic mice produce an anti-depressant like effect, causing a decrease in the mice immobility time in FST.
Conclusion: Metformin pretreatment can decrease depression in cholestatic mice through an NO dependent pathway.

Keywords: Metformin; Nitric Oxide; Cholestasis; Antidepressive Agents; Mice

➢ ***Please cite this paper as:***
Ostadhadi S, Jahanabadi S, Javadi Sh, Saadaei H, Zolfaghari S, Dehpour AR. The Involvement of Nitric Oxide in Antidepressant-Like Effect of Metformin after Bile-Duct Ligation in NMRI Mice. Caspian J Neurol Sci 2015; 1(3):1-10.

Introduction

In some disorders such as diabetes (1) and inflammatory diseases (2) patients can display depressive symptoms. Major depressive disorder (MDD) is a dangerous and heterogeneous disease that has main harmful influences on public health (3, 4). Depression is among the most common mental disorders affecting 120 million people

around the world (5).

Metformin is among the first-line treatments for management of the type 2 diabetes mellitus and avoiding its vascular problems (6). The beneficial properties of metformin have been stated to be mediated via stimulation of AMP-activated protein kinase (AMPK) (7), a protein kinase that is stimulated in response to variations in cellular energy contents (8). AMPK is vital for metabolic homeostasis, which is shared to all eukaryotic cells (9), and can be stimulated by metabolic stresses, such as hypoxia, glucose deficiency and so on (10-12). AMPK activation effect of metformin is not restricted to hepatocytes, but is also detected in other organs such as skeletal muscles, endothelial cells and neurons. AMPK is expressed in the neurons of the developing and adult brain, and more functional brain areas have upper AMPK levels (9). It was revealed that the stimulation of AMPK in the hippocampus of the rats involves in the antidepressant effect of some drug such as ketamine (13). It has been shown that AMPK induces astrocyte stellation, the term used for increasing the outgrowth of neuronal processes, the key feature in depressive disorder pathology (14, 15). Also neuroprotective effects of this antidiabetic drug in central nervous system have been proven (16). On the other hand, interaction between AMPK and nitric oxide pathway has been mentioned in several studies (17, 18). AMPK phosphorylates endothelial isoform of nitric oxide synthase (eNOS), resulting in eNOS activation in a calcium-independent way (6). Neuronal isoform of NOS is also phosphorylated by AMPK (19). NO is a gaseous signaling molecule which has been shown to play the role in some mental functions such as depression (20, 21).

Cholestasis is a kind of liver disease due to structural impairment and dysfunction of hepatobiliary system which at first, results in accumulation of bile acids and other toxins in plasma and liver. At worst, it can leads to hepatic failure and cirrhosis and if untreated ends in death. It was previously showed that in BDL mice immobility time decrease compared to sham-operated animals (22). In other word, AMPK activation in CNS is potently increases in response to bile duct ligation (23). Thus, pharmacological activation of AMPK might provide a new strategy for the management of depression.

Putting all these data together, in this study, we designed an experiment, in which for the first time, we tested the anti-depressive effect of metformin on cholestatic mice. Besides, we examined the mechanism through which metformin exerts it effect. We assumed nitric oxide pathway must, at least in part, be involved in it.

Materials and Methods

1. Animals and Housing Conditions

Male adult NMRI (Naval Medical Research Institute) mice having weight of 20-30 grams obtained from Pasteur Institute were used throughout this study. Two weeks before the behavior experiments, all experimental animals in a group of four to five were placed in plastic cages and were housed under standard laboratory condition of temperature (21-23°C), humidity (55%) and light dark cycle (12-hour light/dark). All animals were permitted to have access to water and food except for the short interval of experiment time. All studies involving animals were done in accordance with the standards and

guidelines of animal care and Council Directive for Care and Use of Laboratory Animals of Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran.

2. Drugs and reagents

The following drugs were utilized during the study: Metformin hydrochloride (Osveh company, Iran), NG-L-arginine methyl ester (L-NAME) (Sigma, Bristol, UK), Aminoguanidine (AG), a specific iNOS inhibitor (Sigma Chemical Co, USA). Usually the drugs were dissolved in water. Drugs were administered through intraperitoneal (i.p.) route in a constant volume of 10 ml/kg body weight.

3. Induction of cholestasis

Mice were divided in three experimental groups. Each group was comprised of 8 to 10 animals: (I) un-operated controls; (II) sham-operated-operated controls; and (III) bile duct ligated (BDL) animals. Briefly, mice were laparotomized under general anesthesia induced by ketamine (50 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). The bile duct was left in place after handling with forceps in sham-operated animals. In BDL animals, the bile duct was double ligated and then the abdominal slit was closed in two layers (24). We evaluated the scratching activity 5 and 7 days after the procedure in BDL and sham-operated animals.

4. Behavioral Tests

4.1. General conditions

All behavioral tests were carried out by an investigator between 08:30 a.m. and 16:00 p.m. Mice were tested every day using one test, which was recorded through video recorder and later it was extrapolated. Also, animals were accustomed to the room for at least one and half an hour before conducting any procedure.

4.2. Open field test

Before performing the forced swimming test (FST), the locomotors behavior was accessed through an open field test (OFT) as described previously (21,25,26). In short, the apparatus consists of a wooden box ($40\times60\times50$ cm^3), the floor arena of which was divided into 12 equal rectangles. Each mouse after placing within the center of the arena, the number of rectangles was counted covered with all paws while moving within the box for at least 6 minutes.

4.3. Forced swimming test

FST is considered as a valid test for the interpretation, assessing of the negative mood like depression. Hence it provides an effective tool for evaluating the efficacy of antidepressants (27). Each mouse was challenged by placing it in a cylindrical tank (30 cm height × 20 cm diameter) filled with tap water having temperature $24\pm1^{\circ C}$, up to 19 cm of height. Mice were permitted to swim freely for 6 minutes. Mice were considered immobile when it became static in the water except those motions which were necessary to hold their head above the water surface. In FST the mice were subjected to swim for 6 minutes, however, the last four minutes of the test were analyzed.

5. Biochemical measurements

Immediately after euthanasia, blood samples were obtained via cardiac puncture (about 0.5 ml/mouse). These experiments were performed by a lab technician blinded to the kind of treatment. Total bilirubin and hepatic enzymes including alanine aminotransferase (ALT) and aspartate transaminase (AST) were assayed biochemically. A commercially available kit

was used to assay these parameters (Zist-Shimi, Tehran, Iran).

6. Experimental design

To distinguish the anti-depressant effects of metformin, distinct doses (25, 50, 200 and 400 mg/kg), were administrated 4 hours before FST to the un-operated animals and 7-days BDL and sham-operated animals. Each group consists of 8 mice. In order to establish the involvement of NO in the antidepressant like effect of metformin, we coinjected the subeffective doses of L-NAME (3 mg/kg) (28) or aminoguanidin (50 mg/kg, i.p.) (29) both with effective dose of metformin (25 mg/kg, i.p.). The L-NAME and amino-guanidin were administered 45 minutes before FST in metformin or saline-treated groups, seven days after bile-duct ligation.

7. Statistical analysis

Alterations in immobility time and locomotor activity in experimental and control groups were done and analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A value of $p<0.05$ was respected to be significant in all groups. All data were stated as the Mean ± SEM. All analyses were performed in SPSS software version 20.

Results

Induction of cholestasis

In current experiment, the mortality rate after the BDL operation was 20% (2 of 10 animals were early sacrificed because poor animal situations obtained). The values of both ALT (555.20 ± 247.47 in BDL *vs.* 63.33 ± 10.03 in sham) and AST (1000.60 ± 560.16 in BDL *vs.* 182.66 ± 45.50 in sham) that denote well-recognized serum indicators of hepatic injury quickly increased 7 days after BDL. Characteristically, the livers of sham-operated animals still look smooth at the end of the test, while the livers of mice that experienced BDL display architectural changes that are principally characterized by the development of edema and fibrotic nodules on the surface of corresponding livers and hydrops of the gall bladder that is filled with large amounts of bile.

Effect of metformin on forced swimming test

In saline injected groups there was no significant alteration in immobility times of mice before procedure, and also between control and sham-operated groups; but as exhibited in figure 1A, the bile duct ligation decreased immobility time compared with sham-operated animals in forced swimming test in seven days after surgical process. Also, there was no significant difference in locomotor activity ($p>0.05$) between above mentioned groups (Figure 1B).

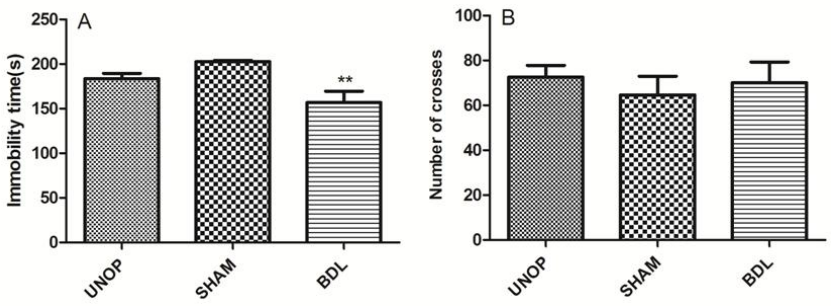

Figure 1: The duration of immobility time in forced-swimming test (A) and the locomotor activity of mice in open field test (B) in unoperated (UNOP), sham-operated (SHOP) and bile-duct ligated (BDL) mice. The groups were tested seven days after bile duct ligation or sham operation. Values are expressed as the Mean±S.E.M. from 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test. $^{**}p<0.01$ compared to the sham-operated and control groups in 7 days studies.

Though in compared with saline treated animals, metformin injection 4 hours before FST did not change the immobility time in unoperated (Figure 2A) and sham-operated (Figure 2B) animals.

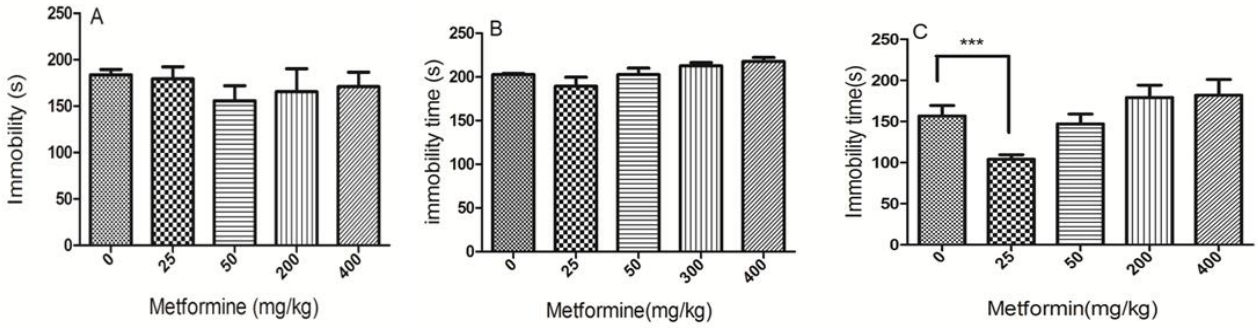

Figure 2: Effect of different doses of metformin (25, 50 , 200 and 400mg/kg, i.p. 4 h before the tests) administration on the immobility time of mice in FST in unoperated (A), sham-operated (B) and bile-duct ligated mice (C). Values are expressed as the Mean±S.E.M. from 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test. ***p<0.001 compared to the sham-operated and control groups in 7 days studies.

The results presented in figure 2C show that metformin at dose 25 mg/kg reduced the immobility time in FST of BDL mice significantly (p<0.001). Furthermore, there was no significant difference in locomotor activity between groups (p>0.05) (Figure 3 A, B,C).

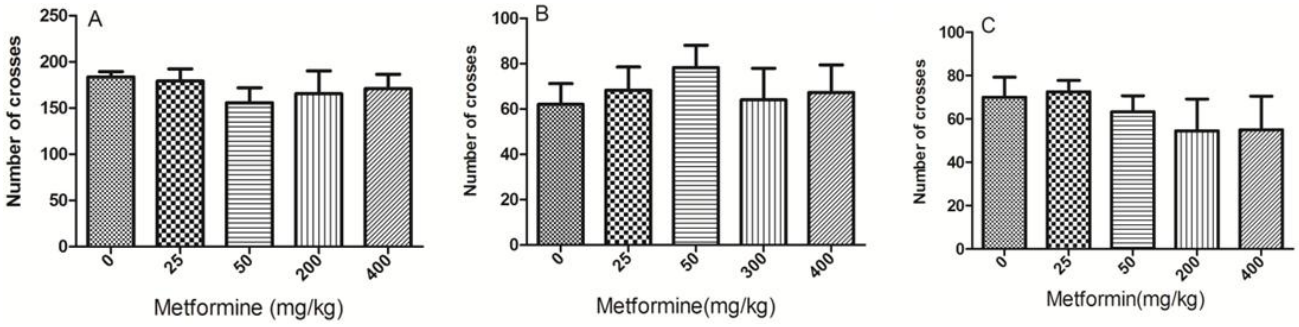

Figure 3: Effect of different doses of metformin (25, 50, 200 and 400mg/kg, i.p. 4 hours before the tests) administration on the locomotor activity of mice in open field test in un-operated (A), sham-operated (B) and bile-duct ligated mice (C). Values are expressed as the Mean±S.E.M. from 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test. *p<0.05 compared to the sham-operated and control groups in 7 days studies.

Effect of l-NAME on anti-immobility effect of metformin on forced-swimming test in BDL animals:

The non-selective NOS inhibitor L-NAME (3 mg/kg, 45 minutes before the tests) did not create significant anti-immobility result (p>0.05, Figure 4A). Injecting this dose of L-NAME did not cause any significant change in the locomotor action of the mice in open-field test (p>0.05) (Figure 4B). As shown in Figure 4A, coadministration of the non-effective dose of L-NAME (3 mg/kg) with effective dose of metformin (25 mg/kg)

inhibited the antidepressant-like effect of metformin in BDL group (p<0.05).

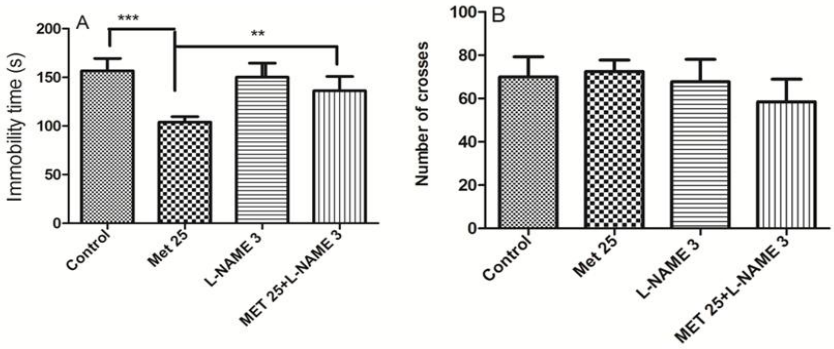

Figure 4: Effect of non-selective nitric oxide synthase (NOS) inhibitor on immobility (A) and locomotor activity (B) of metformin-treated bile-duct ligated mice in FST: Met 25 [metformin (25 mg/kg, effective dose)] was co-administered with the nitric oxide synthase inhibitor L-NAME 3 [L-NAME (3 mg/kg, non-effective dose)]. Values are expressed as the Mean±S.E.M. from 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test. ***p<0.01 compared with the saline-treated control group, **p<0.001 compared with the LNAME 3/ Met 25 group.

Effect of amino-guanidine on anti-immobility effect of metformin on forced swimming test in BDL mice

Amino-guanidine, the selective inhibitor of iNOS, at 50 mg/kg did not display significant anti-immobility effect in FST (Figure 5A) neither did in locomotor activity of BDL group (Figure 5B) (p>0.05). However, when it was injected 45 minutes before the tests in metformin-treated mice, the anti-immobility effect that had previously been viewed by metformin injection was not altered anymore. This means that aminoguanidine cannot prevent the antidepressant effect of metformin (25 mg/kg) in FST of BDL animals (p > 0.05, Figure 5A).

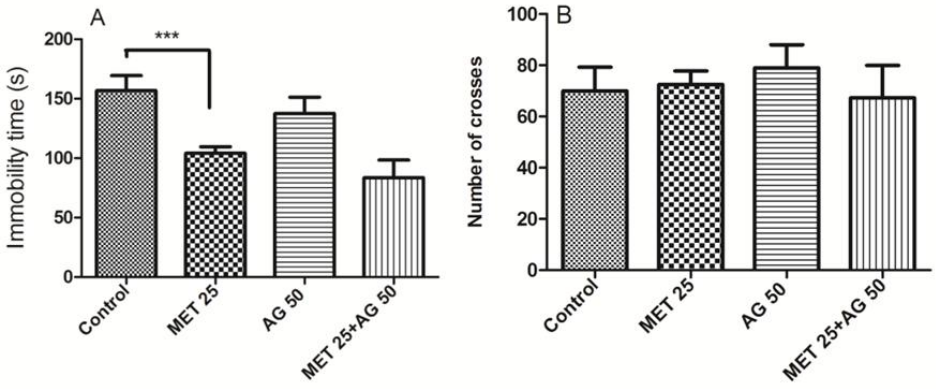

Figure 5: Effect of selective iNOS inhibitor on immobility (A) and locomotor activity (B) of metformin-treated bile-duct ligated mice in FST: Met 25 [metformin (25 mg/kg, effective dose)] was co-administered with the iNOS inhibitor AG 50 [aminoguanidine (50 mg/kg, non-effective dose)]. Values are expressed as the Mean±S.E.M. from 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test. ***p<0.01 compared with the saline-treated group.

Discussion

Compelling mice to swim in a chamber and effort for their life is the main method for the evaluation of agents with antidepressant activity, proposed by Porsolt *et al.* (30, 31). We scrutinized the effect of metformin on this behavioral test in BDL mice. The results of

our study showed a decrease in the immobility time of BDL mice in FST and metformin administration in this group decreased this time additionally. In current experiment the high examined doses was ineffective. The causes for such biphasic dose–response curve are not recognized.

Metformin, a typical and extensively used anti-diabetic drug, stimulates AMPK. It works as an energy controller in all eukaryotic cells (32) and also plays an essential role in connecting metabolism and cancer progress (33). AMPK is stimulated classically by an expansion in the cellular AMP/ATP ratio under situations such as glucose deficiency, hypoxia, ischemia, and heat stress (34). Additionally, it is also stimulated by numerous hormones and cytokines such as adiponectin and leptin and by the anti-diabetic agent metformin (35). Once stimulated, AMPK elevates energy production and restricts energy consumption to guarantee cellular survival (34). AMPK is highly expressed in brain (36) and hippocampal AMPK regulates cognitive role *via* management of neurogenesis and neuroapoptosis (37). Also previous study showed that activation of AMPK involved in anti-depressant effect of some agents such as ketamine (13).

Liver cirrhosis induces malfunctioning brain energy metabolism unrelated to the etiology of the liver disease (23). However, the highest grade of impairment of brain behavior is discovered in patients with liver disorders because low energy supplies in the brain cells of these patients (38). It was exhibited that BDL animals displayed a stable but moderate advancement of AMPK activity compared with the sham group in long period (23). Also in accordance with current experiment our previous study showed that the immobility time significantly reduced after bile-duct ligation in mice (24). To deal with this threat, the brain is armed with protection apparatuses that reply to energy diminution by starting adaptive responses. One such machinery is the AMPK system and pharmacological stimulation of AMPK recovers liver failure-induced brain malfunction (23).

However, there were still some restrictions in the explanation of the consequences. We did not utilize traditional kinase activity analyze to directly evaluated the AMPK activity, though p-AMPK can represent the activity of AMPK, which merits additional examinations.

In next step we showed the involvement of NO in anti-depressant effect of metformin in BDL mice. Metformin has been displayed to enhance the phosphorylation of endothelial (6) and neural (19) NO synthase *via* the AMPK pathway, which in turn augments NO synthase activity and NO levels. In our experiment, the inhibition of NO synthesis inhibited anti-depressant effects of metformin in FST. Previous reports established the role of nitric oxide pathway in the pathophysiology of mood illnesses.

In 1995, Karatinos *et al*. proposed that NO pathway can lead to demonstration of new treatments for numerous neuropsychiatric situations such as mood disorders (39). It was showed that a high plasma content of nitrite, the end product of NO metabolism, has been discovered in depressed patients, indicating to the hypothesis that NO production may enhanced in depression (40).

On the other hand, numerous NOS inhibitors have displayed an antidepressant-like activity in the FST (29, 41). However all this documents proposed that NO production and consequently the serum NO content

enhanced in depression and the NOS inhibitors participated in recovering the mood disorder, there is proof for a dual effect of NO in the FST in mice.

The outcomes of da Silva's report displayed that both the synthesis of NO or the prevention of its synthesis can generate anti-depressant like activity in the FST (20). Also it was showed that in depressed patients, NOx plasma contents decreased compared to healthy persons (42, 43). This means that not only a decreased NO content can produce an antidepressant like activity, but also this outcome could be detected by an enhancement in plasma NO levels. These results explain that the effect of NO content and consequently the influence of L-arginine and NOS inhibitors on the immobility time of mice in FST are dose dependent (20).

To scrutinize the probable role of NO pathway in our investigation, we managed the tests after co-injection of metformin and NOS inhibitors (L-NAME, amino-guanidine). These agents at low doses did not demonstrate significant antidepressant-like effect in FST, but when injected to metformin-treated mice they inhibited the anti-immobility effect of metformin. This unanticipated result displayed that inhibition of NOS can block the effect of metformin, assuming that metformin shows its effect *via* intensifying the NO production. These outcomes propose a role for nNOS in the antidepressant-like effect of metformin, though rejecting involvement of iNOS in this effect.

Conclusion

In conclusion, we have revealed for the first time that the antidepressant-like activity of metformin on BDL mice in FST is mediated by nitric oxide pathway.

Conflict of Interest

No Conflict of interest.

References

1. Hsu YM, Su LT, Chang HM, Sung FC, Lyu SY, Chen PC. Diabetes Mellitus and Risk of Subsequent Depression: a Longitudinal Study. Int J Nurs Stud 2012;49(4):437-44.
2. Nahon S, Lahmek P, Durance C, Olympie A, Lesgourgues B, Colombel JF, et al. Risk Factors of Anxiety and Depression in Inflammatory Bowel Disease. Inflamm Bowel Dis 2012;18(11):2086-91.
3. Baune BT, Adrian I, Jacobi F. Medical Disorders Affect Health Outcome and General Functioning Depending on Comorbid Major Depression in the General Population. J Psychosom Res 2007;62(2):109-18.
4. Kessler RC, Akiskal HS, Ames M, Birnbaum H, Greenberg P, Hirschfeld RM, et al. Prevalence and Effects of Mood Disorders on Work Performance in a Nationally Representative Sample of US Workers. Am J Psychiatry 2006;163(9):1561-8.
5. Hashimoto K. The Role of Glutamate on the Action of Antidepressants. Prog Neuropsychopharmacol Biol Psychiatry 2011;35(7):1558-68.
6. Davis BJ, Xie Z, Viollet B, Zou MH. Activation of the AMP-Activated Kinase by Antidiabetes Drug Metformin Stimulates Nitric Oxide Synthesis *In Vivo* by Promoting the Association of Heat Shock Protein 90 and Endothelial Nitric Oxide Synthase. Diabetes 2006;55(2):496-505.
7. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, et al. Role of AMP-Activated Protein Kinase in Mechanism of Metformin Action. J Clin Invest 2001;108(8):1167-74.
8. Tian R, Musi N, D'Agostino J, Hirshman MF, Goodyear LJ. Increased Adenosine Monophosphate–Activated Protein Kinase Activity in Rat Hearts with Pressure-Overload Hypertrophy. Circulation 2001;104(14):1664-9.
9. Spasić MR, Callaerts P, Norga KK. AMP-Activated Protein Kinase (AMPK) Molecular Crossroad for Metabolic Control and Survival of Neurons. Neuroscientist 2009;15(4):309-16.
10. Culmsee C, Monnig J, Kemp BE, Mattson MP. AMP-Activated Protein Kinase Is Highly Expressed in Neurons in the Developing Rat Brain and Promotes Neuronal Survival

Following Glucose Deprivation. J Mol Neurosci 2001;17(1):45-58.
11. Gadalla AE, Pearson T, Currie AJ, Dale N, Hawley SA, Sheehan M, et al. AICA Riboside Both Activates AMP-Activated Protein Kinase and Competes with Adenosine for the Nucleoside Transporter in the CA1 Region of the Rat Hippocampus. J Neurochem 2004;88(5):1272-82.
12. McCullough LD, Zeng Z, Li H, Landree LE, McFadden J, Ronnett GV. Pharmacological Inhibition of AMP-Activated Protein Kinase Provides Neuroprotection in Stroke. J Biol Chem 2005;280(21):20493-502.
13. Xu SX, Zhou ZQ, Li XM, Ji MH, Zhang GF, Yang JJ. The Activation of Adenosine Monophosphate-Activated Protein Kinase in Rat Hippocampus Contributes to the Rapid Antidepressant Effect of Ketamine. Behav Brain Res 2013;253:305-9.
14. Favero CB, Mandell JW. A Pharmacological Activator of AMP-Activated Protein Kinase (AMPK) Induces Astrocyte Stellation. Brain Res 2007;1168:1-10.
15. Czéh B, Di Benedetto B. Antidepressants Act Directly on Astrocytes: Evidences and Functional Consequences. Eur Neuropsychopharmacol 2013;23(3):171-85.
16. Ullah I, Ullah N, Naseer MI, Lee HY, Kim MO. Neuroprotection with Metformin and Thymoquinone Against Ethanol-Induced Apoptotic Neurodegeneration in Prenatal Rat Cortical Neurons. BMC Neurosci 2012;13(1):11.
17. Li J, Hu X, Selvakumar P, Russell RR, Cushman SW, Holman GD, et al. Role of the Nitric Oxide Pathway in AMPK-Mediated Glucose Uptake and GLUT4 Translocation in Heart Muscle. Am J Physiol Endocrinol Metab 2004;287(5):E834-41.
18. Taleb S, Moghaddas P, Balaei MR, Taleb S, Rahimpour S, Abbasi A, et al. Metformin Improves Skin Flap Survival Through Nitric Oxide System. J Surg Res 2014;192(2):686-91.
19. Chen Z-P, McConell GK, Michell BJ, Snow RJ, Canny BJ, Kemp BE. AMPK Signaling in Contracting Human Skeletal Muscle: Acetyl-CoA Carboxylase and NO Synthase Phosphorylation. Am J Physiol Endocrinol Metab 2000;279(5):E1202-6.
20. da Silva GdL, Matteussi AS, dos Santos ARS, Calixto JB, Rodrigues ALS. Evidence for Dual Effects of Nitric Oxide in the Forced Swimming Test and in the Tail Suspension Test in Mice. Neuroreport 2000;11(17):3699-702.
21. Kordjazy N, Haj-Mirzaian A, Amiri S, Ostadhadi S, Kordjazy M, Sharifzadeh M, et al. Elevated Level of Nitric Oxide Mediates the Anti-Depressant Effect of Rubidium Chloride in Mice. Eur J Pharmacol 2015;762:411-8.
22. Haj-Mirzaian A, Hamzeh N, Javadi-Paydar M, Estakhri MRA, Dehpour AR. Resistance to Depression Through Interference of Opioid and Nitrergic Systems in Bile-Duct Ligated Mice. Eur J Pharmacol 2013;708(1):38-43.
23. Dagon Y, Avraham Y, Ilan Y, Mechoulam R, Berry EM. Cannabinoids Ameliorate Cerebral Dysfunction Following Liver Failure via AMP-Activated Protein Kinase. FASEB J 2007;21(10):2431-41.
24. Haj-Mirzaian A, Hamzeh N, Javadi-Paydar M, Abdollahzadeh Estakhri MR, Dehpour AR. Resistance to Depression through Interference of Opioid and Nitrergic Systems in Bile-Duct Ligated Mice. Eur J Pharmacol 2013;708(1-3):38-43.
25. Haj-Mirzaian A, Kordjazy N, Haj-Mirzaian A, Ostadhadi S, Ghasemi M, Amiri S, et al. Evidence for the Involvement of NMDA Receptors in the Antidepressant-Like Effect of Nicotine in Mouse Forced Swimming and Tail Suspension Tests. Psychopharmacology 2015; [Epub Ahead of Print].
26. Haj-Mirzaian A, Ostadhadi S, Kordjazy N, Dehpour AR, Mehr SE. Opioid/NMDA receptors blockade reverses the depressant-like behavior of foot shock stress in the mouse forced swimming test. European journal of pharmacology. 2014;735:26-31.
27. Petit-Demouliere B, Chenu F, Bourin M. Forced swimming test in mice: a review of antidepressant activity. Psychopharmacology. 2005;177(3):245-55.
28. Harkin AJ, Bruce KH, Craft B, Paul IA. Nitric Oxide Synthase Inhibitors Have Antidepressant-Like Properties in Mice: 1. Acute Treatments Are Active in the Forced Swim Test. Eur J Pharmacol 1999;372(3):207-13.
29. Sadaghiani MS, Javadi-Paydar M, Gharedaghi MH, Fard YY, Dehpour AR. Antidepressant-Like Effect of Pioglitazone in the Forced Swimming Test in Mice: the Role of PPAR-Gamma Receptor and Nitric Oxide Pathway. Behav Brain Res 2011;224(2):336-43.
30. Castagné V, Moser P, Roux S, Porsolt RD. Rodent Models of Depression: Forced Swim and Tail Suspension Behavioral Despair Tests in Rats and Mice. Curr Protoc Neurosci 2011; Chapter 8:Unit 8. 10A.
31. Porsolt R, Bertin A, Jalfre M. Behavioral Despair in Mice: a Primary Screening Test for Antidepressants. Arch Int Pharmacodyn Ther 1977;229(2):327-36.

32. Hardie DG, Carling D, Carlson M. The AMP-Activated/SNF1 Protein Kinase Subfamily: Metabolic Sensors of the Eukaryotic Cell? Annu Rev Biochem 1998;67(1):821-55.
33. Jones RG, Thompson CB. Tumor Suppressors and Cell Metabolism: a Recipe for Cancer Growth. Genes Dev 2009;23(5):537-48.
34. Mihaylova MM, Shaw RJ. The AMPK Signalling Pathway Coordinates Cell Growth, Autophagy and Metabolism. Nat Cell Biol 2011;13(9):1016-23.
35. Shackelford DB, Shaw RJ. The LKB1–AMPK Pathway: Metabolism and Growth Control in Tumour Suppression. Nat Rev Cancer 2009;9(8):563-75.
36. Ronnett GV, Ramamurthy S, Kleman AM, Landree LE, Aja S. AMPK in the Brain: Its Roles in Energy Balance and Neuroprotection. J Neurochem 2009;109(Suppl 1):17-23.
37. Dagon Y, Avraham Y, Magen I, Gertler A, Ben-Hur T, Berry EM. Nutritional Status, Cognition, and Survival: a New Role for Leptin and AMP Kinase. J Biol Chem 2005;280(51):42142-8.
38. Barbiroli B, Gaiani S, Lodi R, Iotti S, Tonon C, Clementi V, et al. Abnormal Brain Energy Metabolism Shown by *In Vivo* Phosphorus Magnetic Resonance Spectroscopy in Patients with Chronic Liver Disease. Brain Res Bull 2002;59(1):75-82.
39. Karatinos J, Rosse RB, Deutsch SI. The Nitric Oxide Pathway: Potential Implications for Treatment of Neuropsychiatric Disorders. Clin Neuropharmacol 1995;18(6):482-99.
40. Suzuki E, Yagi G, Nakaki T, Kanba S, Asai M. Elevated Plasma Nitrate Levels in Depressive States. J Affect Disord 2001;63(1-3):221-4.
41. Ghasemi M, Sadeghipour H, Mosleh A, Sadeghipour HR, Mani AR, Dehpour AR. Nitric Oxide Involvement in the Antidepressant-Like Effects of Acute Lithium Administration in the Mouse Forced Swimming Test. Eur Neuropsychopharmacol 2008;18(5):323-32.
42. Chrapko WE, Jurasz P, Radomski MW, Lara N, Archer SL, Le Mellédo JM. Decreased Platelet Nitric Oxide Synthase Activity and Plasma Nitric Oxide Metabolites in Major Depressive Disorder. Biol Psychiatry 2004;56(2):129-34.
43. Selley ML. Increased (E)-4-Hydroxy-2-Nonenal and Asymmetric Dimethylarginine Concentrations and Decreased Nitric Oxide Concentrations in the Plasma of Patients with Major Depression. J Affect Disord 2004;80(2-3):249-56.