Ghrelin Alleviates MDMA-Induced Disturbance of Serum Glucose and Lipids Levels in the Rat

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Received: 03 May 2017; Accepted: 22 Jul. 2017

Abstract- Hepatotoxicity is one of the clinically adverse effects of ecstasy (3, 4-methylenedioxymethamphetamine; MDMA) consumption. The detoxification tissue, liver, plays a central role in maintaining circulating levels of glucose and lipid. Hypoglycemia and hypotriglyceridemia have been reported due to ecstasy abuse. Ghrelin is a 28-amino-acid peptide secreted predominantly from the stomach. It has been demonstrated that ghrelin has hepatoprotective effects and is able to increase blood glucose concentration. In the current study, we explored the effect of hepatotoxic dose of MDMA and therapeutic use of exogenous ghrelin on the serum levels of glucose and lipids in four groups of rats. MDMA caused a severe and transient reduction in circulating levels of glucose and triglyceride and increased serum LDL. However, cholesterol and HDL levels remained unchanged. Meanwhile, altered hepatic architecture was observed with intracellular vacuolation that may indicate intracellular accumulation of lipid droplets. In addition, following ghrelin administration, the blood sugar levels improved and LDL levels returned to the baseline value, and ghrelin treatment did not improve triglycerides levels. These results showed that MDMA causes hypoglycemia, hypotriglyceridemia, and hyper LDL-cholesterolemia. To our knowledge, this is the first report showing ghrelin administration could improve hypoglycemia and normalize LDL levels induced by MDMA and partially restore hepatic architecture.

Acta Med Iran 2017;55(12):736-743.

Keywords: Hepatotoxicity; 3,4-methylenedioxymethamphetamine; Ghrelin; Glucose; Triglyceride; LDL

Introduction

Ecstasy is the popular name of the recreational drug 3, 4-methylenedioxymethamphetamine (MDMA) (1,2). Despite the deleterious effects associated with its use, MDMA is being consumed worldwide especially among young people for their euphoric and stimulant effects (3,4). MDMA is identified as a hepatotoxic agent for humans (5), and interestingly, the MDMA-induced hepatic damage has been identified as the cause of death among some consumers (6-9). Reduction in blood glucose (10,11) and triglycerides (12) levels have been reported due to MDMA. The liver plays a central role in maintaining circulating glucose levels by balancing the uptake and storage of glucose (13). Liver as a key metabolic organ plays an important role in various aspects of lipid metabolism (14). However, the precise role of MDMA-induced hepatotoxicity in promoting metabolic disturbances is yet not clear.

Ghrelin is a 28-amino-acid peptide secreted predominantly from X/A-like enteroendocrine cells of the stomach (15,16). The major physiological action of ghrelin is to stimulate growth hormone (GH) secretion (17). Moreover, this orexigenic peptide regulates a wide array of physiological actions including energy balance, appetite, food intake, glucose hemostasis and long-term regulation of body weight (15,16). Ghrelin stimulates appetite and participates in increased blood glucose concentration (16). It has been demonstrated that acute administration of MDMA causes a transient increase in serum ghrelin level with no considerable change in the GH levels (18). Numerous studies have supported the

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hepatoprotective effect of ghrelin (19-21). In addition, ghrelin has been shown to reduce hepatic fatty degeneration and increase blood glucose level in acute liver injury induced by carbon tetrachloride (22). Therefore, this study was undertaken to evaluate the efficacy of exogenous ghrelin treatment on the alleviation of MDMA-induced changes in serum levels of glucose and lipids in rats.

Materials and Methods

Materials

MDMA was procured from the organic chemistry laboratory of Faculty of Pharmacy (Tehran University of Medical Sciences). Rat acyl ghrelin was purchased from Sigma Aldrich (St. Louis, MO, USA). Reagent for measurement of glucose concentration was purchased from BioSystems S.A. (Barcelona, Spain). Specific kits for cholesterol, triglyceride, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) measurement were purchased from (Bionik, Iran).

Animals

Forty-eight adult male Wistar rats weighing 200-250 g were obtained from the animal house at Department of Physiology (Tehran University of Medical Sciences, Iran). The rats were maintained in regular cages under the controlled environmental conditions (20±2˚ C and 12 h light-dark cycle) and allowed free access to standard lab chow and water. All experimental procedures were in accordance with the Guidelines for the Care and Use of Laboratory Animals published by National Institutes of Health (NIH Publication No. 85-23, revised 1996) and approved by the Research Council of the Tehran University of Medical Sciences.

Experimental protocol

Animals were randomly divided into four groups (n=12, each group); 1) Control group received normal saline *i.p.* at 08:00 *a.m.* 2) MDMA group received single dose of MDMA 20 mg/kg; *i.p.* at 08:00 *a.m.* 3) Ghrelin group received 2 doses of 10 nmol/kg of ghrelin dissolved in normal saline *(i.p.)* 3 h apart. 4) MDMA+ghrelin group received MDMA (20 mg/kg; *i.p.)* at 08:00 *a.m.* and 1 h later received ghrelin (10 nmol/kg; *i.p.*) similar to ghrelin group. For assessment of various parameters, animals were euthanized 6 h and 24 h following the intervention. In order to determine the appropriate MDMA dosage, we carried out a dose-response study and accordingly, 20 mg/kg was chosen. Except for our dose-response study, the same dose has also been used in earlier studies (18,23,24). Ghrelin dose was obtained from a previous study of our lab (25).

Blood biochemistry studies

The whole blood was taken by heart puncture and centrifuged at 3000 RPM for 10 min. The serum was separated and stored at -70° C. Blood sugar, cholesterol, triglyceride, LDL, and HDL levels were determined using autoanalyzer Hitachi 912. Serum levels of glucose were measured by the glucose oxidase-peroxidase method on Hitachi 912. Cholesterol and triglyceride levels were assessed using enzymatic-colorimetric CHOD-POD and GPO-POD method, respectively on Hitachi 912. The direct enzymatic colorimetric method was used for the quantitative measurement of LDL and HDL on Hitachi 912.

Data analysis

Data are represented as mean±SEM. The normality of the data was assessed by the Kolmogorov-Smirnov test. One-way ANOVA and post-hoc *Tukey* test were used to compare the means between different groups. Differences were considered statistically significant at $P<0.05$.

Histological study

Liver tissue was excised under deep anesthesia and immersed in 10% buffered formalin. After paraffin embedding and sectioning of tissue into 5 μm slices, all sections were stained with Hematoxylin and Eosin (H and E). Histological evaluation was performed by using light microscopy in a blinded manner.

Results

General consideration

We surveyed the effect of MDMA and therapeutic effect of ghrelin on blood sugar and lipids by collecting blood 6 h and 24 h after MDMA administration.

Selection of MDMA dose

As a first step before starting our study, we performed a dose-response study to determine the optimal dose of MDMA. Since transaminase levels are the most reliable indicators of hepatic injury (26), in our study, concentrations of ALT and AST were used as reliable markers for detecting liver damage (27). Serum levels of ALT and AST measured 6 and 24 h after a single dose of *i.p.* injection of MDMA (5, 10, 20 or 40 mg/kg) or saline. The dose of 40 mg/kg has a mortality rate of 50%. Thus we removed this group of the animal

before serum transaminase measurement. Significant increase in ALT and AST levels observed 6 h post-MDMA injection. Accordingly, this MDMA dose selected for our study. Dose-response study data are shown in Table 1.

Table 1. Serum ALT and AST levels in control animals and MDMA (5, 10 and 20 mg/kg)-injected animals

Groups	ALT(6 h)	AST(6 h)	ALT(24 h)	AST(24 h)
Control	45.66 ± 2.6	189.66 ± 30.8	45.66±2.6	189.66±30.8
MDMA 5 mg/kg	52.50 ± 2.7	165.25 ± 7.3	75.75±4.36	209.25±19.53
MDMA 10 mg/kg	60.66 ± 9.2	221.66 ± 18.26	70.66 ± 4.17	229.33 ± 20.62
MDMA 20 mg/kg	106.66 ± 16.76*#	675.16 ± 162.95#	113.37 ± 22.41	621.00 ± 185.88

Levels of blood transaminases were measured 6 h and 24 h after the intervention. * P<0.05 as compared to control group; # P<0.05 as compared to MDMA 5 mg/kg group (n=3-6)

The effects of MDMA and treatment with ghrelin on serum glucose level

Blood sugar of the MDMA-treated rats six hours after the intervention was significantly lower (P<0.001) and was less significant in MDMA+Ghrelin group (P<0.01) versus the control group. Meanwhile, 24 hours after MDMA administration, blood sugar showed no significant changes in MDMA and MDMA+Ghrelin as compared to the control group (Figure 1).

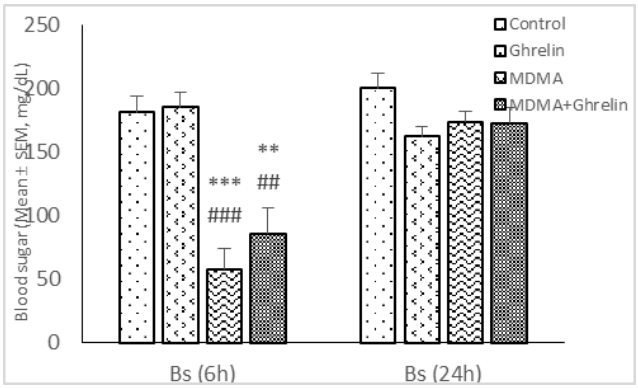

Figure 1. Serum sugar levels in control animals, ghrelin (10 nmol/kg, *i.p.* 3 h apart)-injected animals, MDMA (20 mg/kg, *i.p.*)-injected animals and MDMA in combination with ghrelin. Levels of blood sugar were measured 6 and 24 h after the intervention. ** P<0.01, *** P<0.001 as compared to control group; ## P<0.01, ### P<0.001 as compared to ghrelin group (n = 6)

The effects of MDMA and treatment with ghrelin on triglyceride and cholesterol levels

The blood cholesterol level did not change after MDMA injection and therapeutic use of ghrelin (Figure 2). The level of triglyceride in MDMA and MDMA+Ghrelin groups significantly decreased versus control (P<0.001 for both cases) and ghrelin (P<0.01 for both cases) groups (Figure 3).

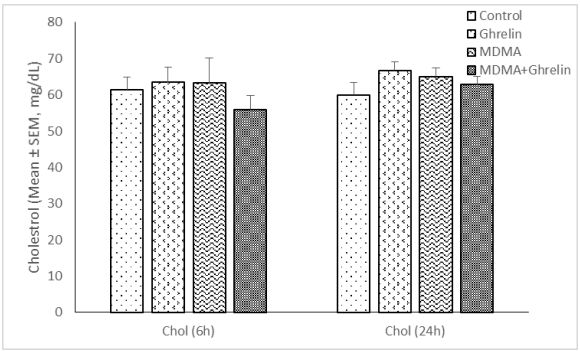

Figure 2. Serum cholesterol levels in control animals, ghrelin (10 nmol/kg, *i.p.* 3 h apart)-injected animals, MDMA (20 mg/kg, *i.p.*)-injected animals and MDMA in combination with ghrelin. Levels of cholesterol were measured 6 and 24 h after intervention

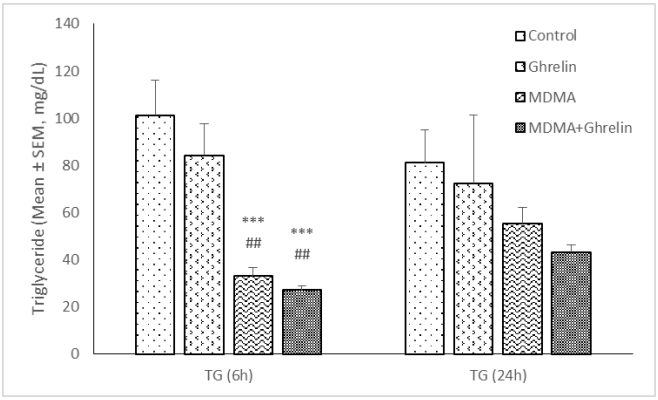

Figure 3. Serum triglyceride levels in control animals, ghrelin (10 nmol/kg, *i.p.* 3 h apart)- injected animals, MDMA (20 mg/kg, *i.p.*)-injected animals and MDMA in combination with ghrelin. Levels of triglyceride were measured 6 and 24 h after the intervention. *** $P<0.001$ as compared to control group; ## $P<0.01$ as compared to ghrelin group (n = 6)

The effects of MDMA and treatment with ghrelin on HDL and LDL cholesterol levels

There was no significant alteration in serum HDL level between groups (Figure 4). In contrast, LDL level significantly increased in MDMA group at 6 and 24 hours post-intervention ($P<0.01$, $P<0.05$, respectively) versus control. Treatment of MDMA group with ghrelin caused a significant reduction of serum LDL ($P<0.05$) as compared to MDMA group at 6 hours after intervention. Furthermore, there was no significant difference between MDMA+Ghrelin group and control group at any time (Figure 5).

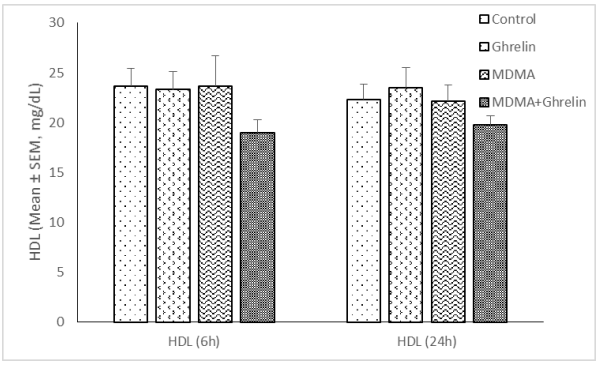

Figure 4. Serum high-density lipoprotein (HDL) levels in control animals, ghrelin (10 nmol/kg, *i.p.* 3 h apart)-injected animals, MDMA (20 mg/kg, *i.p.*)-injected animals and MDMA in combination with ghrelin. Levels of HDL were measured 6 and 24 h after intervention (n = 6)

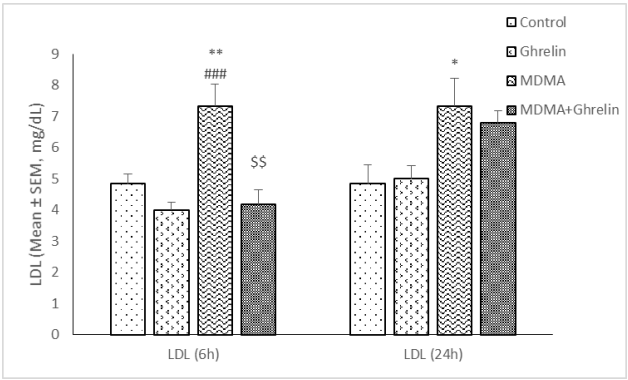

Figure 5. Serum low-density lipoprotein (LDL) levels in control animals, ghrelin (10 nmol/kg, *i.p.* 3 h apart)-injected animals, MDMA (20 mg/kg, *i.p.*)-injected animals and MDMA in combination with ghrelin. Levels of LDL were measured 6 and 24 h after intervention. ** $P < 0.01$ as compared to control group; ### $P < 0.001$ as compared to ghrelin group; $$ $P < 0.01$ as compared to MDMA group (n = 6)

Histological findings

Microscopic evaluation of the liver tissue section stained with H and E showed that in control and ghrelin-treated control groups, a normal hepatic architecture was observed and hepatocytes had round nuclei with condensed chromatin and the cells were radially arranged around central vein. Acidophilic staining intensity of the cytoplasm may indicate the presence of glycogen. Additionally, in MDMA group, altered hepatic architecture was observed with intracellular vacuolation that may indicate intracellular accumulation of lipid droplets. The cytoplasmic stain of liver sections in MDMA group displayed glycogen stores depletion as compared to control group. Furthermore, ghrelin treatment of MDMA group attenuated all of these inappropriate alterations (Figure 6).

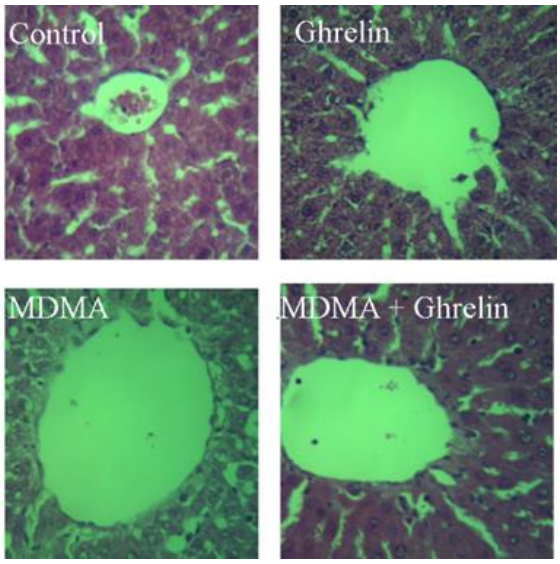

Figure 6. Histological sections of the rat liver from levels in control animals, ghrelin (10 nmol/kg, *i.p.* 3 h apart)-injected animals, MDMA (20 mg/kg, *i.p.*)-injected animals and MDMA in combination with ghrelin.were stained with Hematoxylin and Eosin stain

Discussion

Our findings demonstrate that the OBQ-44 clearly Hepatotoxicity is one of the adverse effects of MDMA consumption (8,10,28). The liver plays central role in lipid (29) and glucose homeostasis in the body. In this regard, the present study demonstrated a severe and transient reduction in circulating levels of glucose and triglyceride following MDMA administration. Moreover, we showed lasting increase in serum LDL at least up to 24 hours after drug injection in rats. Moreover, we observed a transient increase in liver transaminase levels (data not shown). We demonstrated that following the therapeutic administration of ghrelin, the blood sugar levels improved and LDL levels returned to the baseline value. However, ghrelin treatment did not affect levels of circulating triglycerides.

In consistent with our results, it has been reported that blood glucose levels decrease after MDMA administration in animal models (10,28). MDMA-induced liver toxicity can be attributed to reduction in glucose levels following MDMA exposure. This effect resembles those seen in two separate case report studies in which early hypoglycemia was detected after MDMA intoxication (30,31). One of them fainted and was in a minimally responsive state (30) and the other collapsed after undergoing a grand-mal convulsion (31). In these cases, MDMA-induced hypoglycemia required intravenous infusion of high-dose dextrose solutions. It is interesting to note that the MDMA hepatotoxicity is likely a cause of hypoglycemia and altered mental status. Beitia *et al.*, demonstrated that liver glycogen content after MDMA treatment markedly reduced. They found approximately 83% reduction in liver glycogen content which was accompanied with a significant decrease in blood glucose level (10). In the current study, animals that received ghrelin after MDMA injection showed less decline in blood glucose level than those received MDMA. Ghrelin may have contributed to improve blood glucose via stimulation of liver glycogenolysis and neoglucogenesis (16). Thus, the increase in blood glucose level observed in ghrelin-treated subjects cannot be attributed to compensatory

increase in liver glycogenolysis but may be ascribed to enhanced neoglucogenesis. However, the question of how MDMA-induced reduction in circulating blood levels of glucose remains unanswered

Liver is vital for synthesizing circulating lipids and lipoproteins and is considered as a central organ for lipid metabolism in the creatures (29). Our results showed that even a single dose of MDMA could alter lipid and lipoprotein synthesis. MDMA-induced hypotriglyceridemia is consistent with the result of Kwack *et al.*, who reported reduced levels of triglycerides in male mice treated with MDMA for 28 days (12). Moreover, a case report study indicates low cholesterol and triglyceride levels in a patient undergoing MDMA-induced acute myocardial infarction (32). Furthermore, the hepatic cholesterol and triglycerides levels have been shown to increase in MDMA-exposed animals (10,27). Inhibition of 3-ketoacyl-CoA thiolase activity involved in β-oxidation pathway was the likely cause of hepatic fat accumulation following MDMA exposure (27). The current experimental results revealed MDMA-induced reduction in circulating triglycerides. These findings may be as a consequence of inactivation of transferring enzymes responsible for exportation of lipid from liver into the blood. Further studies are warranted to investigate the potential molecular mechanism that may contribute to MDMA-induced reductions in circulatory triglyceride levels.

Plasma LDL levels are mainly determined by the liver (33). The level of circulating LDL levels depends on events taking place in the liver. Liver clears approximately 70% of circulating LDL via LDL receptors. Indeed, the number of LDL receptors in the liver determines the circulating LDL levels as they control the rate of LDL production and clearance. Low LDL receptor activity results in an increase in LDL production and decrease in LDL clearance leading to a rise in plasma LDL levels. Levels of hepatic LDL receptors are regulated by the amount of cholesterol in the cell (33,34). Taken together, these findings suggest that increased hepatic cholesterol is likely to decrease hepatic LDL receptors and consequently elevate circulatory levels of LDL. However, more studies are needed to confirm this hypothesis and to understand the exact molecular mechanism responsible for MDMA-induced alterations in LDL levels. It has been demonstrated that ghrelin may influence lipid metabolism by affecting the liver and adipose tissues (16). To our knowledge, this is the first report demonstrating the beneficial effect of ghrelin against the MDMA-induced rise in LDL levels. The present study is in accordance with the findings of Zwirska-Korczala *et al.*, who reported a negative correlation between acylated ghrelin and circulating levels of LDL (35). Some studies have suggested a profound role of ghrelin in mediating compensatory mechanisms to maintain metabolic balance (36). The mechanisms by which MDMA leads to the development of metabolic disorders have not been completely elucidated yet.

Our present study showed MDMA causes dyslipidemia characterized by high LDL cholesterol and low triglyceride that were associated with hypoglycemia. To the best of our knowledge, this is the first report to demonstrate that exogenous ghrelin can improve hypoglycemia and normalize LDL levels induced by MDMA and could prevent alterations of hepatic architecture.

Acknowledgements

We would like to acknowledge the financial support from the Research Vice-chancellor of Tehran University of Medical Sciences (grant number no: 28325).

References

1. Bershad AK, Miller MA, Baggott MJ, de Wit H. The effects of MDMA on socio-emotional processing: Does MDMA differ from other stimulants? J Psychopharmacol 2016;30:1248-58.
2. Ferraz-de-Paula V, Ribeiro A, Souza-Queiroz J, Pinheiro M, Vecina J, Souza D, et al. 3, 4-Methylenedioxymethamphetamine (MDMA–Ecstasy) decreases neutrophil activity through the glucocorticoid pathway and impairs host resistance to Listeria monocytogenes infection in mice. J Neuroimmune Pharmacol 2014;9:690-702.
3. Karch SB. A historical review of MDMA. Open Forensic Sci J 2011;4:20-4.
4. Steinkellner T, Freissmuth M, Sitte HH, Montgomery T. The ugly side of amphetamines: short-and long-term toxicity of 3, 4-methylenedioxymethamphetamine (MDMA,'Ecstasy'), methamphetamine and D-amphetamine. Biol Chem 2011;392:103-15.
5. Carvalho M, Pontes H, Remião F, L Bastos M, Carvalho F. Mechanisms underlying the hepatotoxic effects of ecstasy. Curr Pharm Biotechnol 2010;11:476-95.
6. Ellis A, Wendon J, Portmann B, Williams R. Acute liver damage and ecstasy ingestion. Gut 1996;38:454-8.
7. Henry J, Jeffreys K, Dawling S. Toxicity and deaths from 3, 4-methylenedioxymethamphetamine (" ecstasy").

Lancet 1992;340:384-7.
8. Garbino J, Henry J, Mentha G, Romand J-A. Ecstasy ingestion and fulminant hepatic failure: liver transplantation to be considered as a last therapeutic option. Vet Hum Toxicol 2001;43 :99-102.
9. Caballero F, Lopez-Navidad A, Cotorruelo J, Txoperena G. Ecstasy-induced brain death and acute hepatocellular failure: multiorgan donor and liver transplantation. Transplantation 2002;74:532-7.
10. Beitia G, Cobreros A, Sainz L, Cenarruzabeitia E. Ecstasy-induced toxicity in rat liver. Liver 2000;20:8-15.
11. Andreu V, Mas A, Bruguera M, Salmerón JM, Moreno V, Nogué S, et al. Ecstasy: a common cause of severe acute hepatotoxicity. J Hepatol 1998;29:394-7.
12. Kwack SJ, Yoon KS, Lim SK, Gwak H-m, Kim JY, Um YM, et al. A one-generation reproductive toxicity study of 3, 4-methylenedioxy-n-methamphetamine (MDMA, Ecstasy), an amphetamine derivative, in C57BL/6 mice. J Toxicol Environ Health A 2014;77:1431-42.
13. Nordlie RC, Foster JD, Lange AJ. Regulation of glucose production by the liver. Annu Rev Nutr 1999;19:379-406.
14. Canbay A, Bechmann L, Gerken G. Lipid metabolism in the liver. Z Gastroenterol 2007;45:35-41.
15. Dixit VD, Schaffer EM, Pyle RS, Collins GD, Sakthivel SK, Palaniappan R, et al. Ghrelin inhibits leptin-and activation-induced proinflammatory cytokine expression by human monocytes and T cells. J Clin Invest 2004;114:57-66.
16. Delporte C. Structure and physiological actions of ghrelin. Scientifica (Cairo). 2013;2013.
17. Kojima M, Hosoda H, Matsuo H, Kangawa K. Ghrelin: discovery of the natural endogenous ligand for the growth hormone secretagogue receptor. Trends Endocrinol Metab 2001;12:118-22.
18. Kobeissy FH, Jeung JA, Warren MW, Geier JE, Gold MS. PRECLINICAL STUDY: Changes in leptin, ghrelin, growth hormone and neuropeptide-Y after an acute model of MDMA and methamphetamine exposure in rats. Addict Biol 2008;13:15-25.
19. İşeri SÖ, Şener G, Saglam B, Ercan F, Gedik N, Yeğen BÇ. Ghrelin alleviates biliary obstruction-induced chronic hepatic injury in rats. Regul Pept 2008;146:73-9.
20. Moreno M, Chaves JF, Sancho-Bru P, Ramalho F, Ramalho LN, Mansego ML, et al. Ghrelin attenuates hepatocellular injury and liver fibrogenesis in rodents and influences fibrosis progression in humans. Hepatology 2010;51:974-85.
21. Li Y, Hai J, Li L, Chen X, Peng H, Cao M, et al. Administration of ghrelin improves inflammation, oxidative stress, and apoptosis during and after non-alcoholic fatty liver disease development. Endocrine 2013;43:376-86.
22. Çetin E, Kanbur M, Çetin N, Eraslan G, Atasever A. Hepatoprotective effect of ghrelin on carbon tetrachloride-induced acute liver injury in rats. Regul Pept 2011;171:1-5.
23. Cerretani D, Bello S, Cantatore S, Fiaschi A, Montefrancesco G, Neri M, et al. Acute administration of 3, 4-methylenedioxymethamphetamine (MDMA) induces oxidative stress, lipoperoxidation and TNFα-mediated apoptosis in rat liver. Pharmacol Res 2011;64:517-27.
24. Aguirre N, Barrionuevo M, Ramírez MJ, Del Río J, Lasheras B. α-Lipoic acid prevents 3, 4-methylenedioxy-methamphetamine (MDMA)-induced neurotoxicity. Neuroreport 1999;10:3675-80.
25. Jahromi MG, Nabavizadeh F, Vahedian J, Nahrevanian H, Dehpour A-R, Zare-Mehrjardi A. Protective effect of ghrelin on acetaminophen-induced liver injury in rat. Peptides 2010;31:2114-7.
26. Giboney PT. Mildly elevated liver transaminase levels in the asymptomatic patient. Am Fam Physician 2005;71:1105-10.
27. Moon KH, Upreti VV, Yu LR, Lee IJ, Ye X, Eddington ND, et al. Mechanism of 3, 4-methylenedioxymethamphetamine (MDMA, ecstasy)-mediated mitochondrial dysfunction in rat liver. Proteomics 2008;8:3906-18.
28. Soto-Montenegro M, Vaquero JJ, Arango C, Ricaurte G, Garcia-Barreno P, Desco M. Effects of MDMA on blood glucose levels and brain glucose metabolism. Eur J Nucl Med Mol Imaging 2007;34:916-25.
29. Boemeke L, Bassani L, Marroni CA, Gottschall CBA. Lipid profile in cirrhotic patients and its relation to clinical outcome. Arq Bras Cir Dig 2015;28:132-5.
30. Carrera P, Iyer VN. Profound hypoglycemia with ecstasy intoxication. Case Rep Emerg Med 2015;2015.
31. Montgomery H, Myerson S. 3, 4-methylenedioxymethamphetamine (MDMA, or "ecstasy") and associated hypoglycemia. Am J Emerg Med 1997;15:218.
32. Lai T-I, Hwang J-J, Fang C-C, Chen W-J. Methylene 3, 4 dioxymethamphetamine–induced acute myocardial infarction. Ann Emerg Med 2003;42:759-62.
33. Dietschy JM, Turley SD, Spady DK. Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. J Lipid Res 1993;34:1637-59.
34. Feingold KR, Grunfeld C. Introduction to lipids and lipoproteins. 2015.
35. Zwirska-Korczala K, Konturek S, Sodowski M, Wylezol M, Kuka D, Sowa P, et al. Basal and postprandial plasma levels of Pyy, ghrelin, cholecystokinin, gastrin and insulin

in women with moderate and morbid obesity and metabolic syndrome. J Physiol Pharmacol 2007;58:13-35.

36. Ma S, Ge Y, Gai X, Xue M, Li N, Kang J, et al. Transgenic n-3 PUFAs enrichment leads to weight loss via modulating neuropeptides in hypothalamus. Neurosci Lett.2016;611:28-32.