Alcohol effects on metabolism via acetyl-Coenzyme A synthetase: systematic review and meta-analysis

Efeitos do álcool sobre o metabolismo da via da acetil-Coenzima A sintetase: revisão sistemática e metanálise

Efectos del alcohol sobre el metabolismo a través de la acetil-Coenzima A sintetasa: revisión sistemática y metanálisis

Gabriel Souza-Silva[1], Marcos Paulo Felipe[1], Pollyanna Vieira Gomes da Silva[2], Andrea Frozino Ribeiro[1]*.

ABSTRACT

Objective: To evaluate the effects of alcohol on the metabolism of the acetyl-Coenzyme A synthetase pathway. **Methods:** For this meta-analysis, articles published up to December 2019 were searched and the levels of phosphorylated adenosine, acetate, acetyl-CoA, acetoacetate and acetyl-CoA activity were evaluated. The standardized mean difference and the weighted mean from the meta-analysis were compared using the random model. **Results:** Alcohol promoted an increase in the levels of adenosine triphosphate, adenosine diphosphate, adenosine monophosphate and inorganic phosphate (odds ratio [OR] = 1.16; 95% confidence interval [CI], 0.42, 2 .10; p = 0.003). In addition, the levels of acetate, acetoacetate and acetyl-CoA increased due to alcohol (OR = 2.05; 95% CI, 1.07, 3.04; p < 0.0001), however, with high heterogeneity (I^2 = 77%). Acetyl-CoA synthetase (ACSS2) activity increased after alcohol administration (OR = 2.07; 95% CI, 0.16, 3.97; p = 0.03), with high heterogeneity (I^2 = 88%). **Final considerations:** Alcohol has a supplementary effect on this metabolic pathway, but in a restricted way. Furthermore, ACSS2 activity increased after exposure to alcohol. However, these effects of alcohol were time dependent.

Keywords: Alcoholism, Adenosine, Ethanol, Acetyl coenzyme A, Allostasis.

RESUMO

Objetivo: Avaliar os efeitos do álcool sobre o metabolismo da via da acetil-Coenzima A sintetase. **Métodos:** Para esta metanálise, foram pesquisados por artigos publicados até dezembro de 2019 e avaliados os níveis de adenosina fosforilada, acetato, acetil-CoA, acetoacetato e a atividade de acetil-CoA. A diferença média padronizada e a média ponderada da metanálise foram comparadas usando o modelo aleatório. **Resultados:** O álcool promoveu um aumento nos níveis de trifosfato de adenosina, difosfato de adenosina, monofosfato de adenosina e fosfato inorgânico (odds ratio [OR] = 1,16; intervalo de confiança de 95% [IC], 0,42, 2,10; p = 0,003). Além disso, os níveis de acetato, acetoacetato e acetil-CoA aumentaram devido ao álcool (OR = 2,05; IC 95%, 1,07, 3,04; p < 0,0001), porém, com alta heterogeneidade (I^2 = 77%). A atividade da acetil-CoA sintetase (ACSS2) apresentou aumento após administração de álcool (OR = 2,07; IC 95%, 0,16, 3,97; p = 0,03), com elevada heterogeneidade (I^2 = 88%). **Considerações finais:** O álcool tem um efeito de suplemento nesta via metabólica, mas de forma restrita. Além disso, a atividade ACSS2 aumentou após a exposição ao álcool. No entanto, esses efeitos do álcool eram dependentes do tempo.

Palavras-chave: Alcoolismo, Adenosina, Etanol, Acetilcoenzima A, Alostase.

RESUMEN

Objetivo: Evaluar los efectos del alcohol en el metabolismo de la vía de la acetil-Coenzima A sintetasa. **Métodos:** Para este metanálisis, se buscaron artículos publicados hasta diciembre de 2019 y se evaluaron los niveles de actividad de adenosina fosforilada, acetato, acetil-CoA, acetoacetato y acetil-CoA. La diferencia de medias estandarizada y la media ponderada del metanálisis se compararon mediante el modelo aleatorio. **Resultados:** El alcohol promovió un aumento en los niveles de trifosfato de adenosina, difosfato de

[1] Pontifícia Universidade Católica de Minas Gerais (PUC Minas), Betim - MG.
*E-mail: andreafroz@pucminas.br
[2] Universidade Federal de Minas Gerais (UFMG), Belo Horizonte - MG.

SUBMETIDO EM: 4/2022 | **ACEITO EM: 4/2022** | **PUBLICADO EM: 4/2022**

adenosina, monofosfato de adenosina y fosfato inorgánico (odds ratio [OR] = 1,16; intervalo de confianza [IC] del 95%, 0,42, 2,10; p = 0,003). Además, los niveles de acetato, acetoacetato y acetil-CoA aumentaron debido al alcohol (OR = 2,05; IC 95%, 1,07, 3,04; p < 0,0001), sin embargo, con alta heterogeneidad (I^2 = 77%). La actividad de la acetil-CoA sintetasa (ACSS2) aumentó después de la administración de alcohol (OR = 2,07; IC del 95 %, 0,16; 3,97; p = 0,03), con alta heterogeneidad (I^2 = 88 %). **Consideraciones finales:** El alcohol tiene un efecto suplementario sobre esta vía metabólica, pero de forma restringida. Además, la actividad de ACSS2 aumentó después de la exposición al alcohol. Sin embargo, estos efectos del alcohol dependían del tiempo.

Palabras clave: Alcoholismo, Adenosina, Etanol, Acetilcoenzima A, Alostasis.

INTRODUCTION

In most parts of the world, the consumption of psychoactive substances such as alcohol is socially accepted for recreational purposes. Nevertheless, distinguishing the boundary between use and abuse is a continuous challenge. According to the World Health Organization (WHO), the problems associated with alcohol use are consequence of multiple factors that bring damage to health, and abuse and dependence being the most impactful, causing increased morbidity and mortality rates in the world population (WHO, 2018).

In 2018, the global average consumption of alcohol, aged 15 and over, was 6.18 liters per person. As a comparative measure, traditional wine contains about 12% pure alcohol in its composition, so a liter of wine contains 0.12 liters of pure alcohol. Therefore, a global average of 6.2 liters of pure alcohol is equivalent to 53 bottles of wine per person per year, approximately 1 liter of wine per week. This consumption varies across the world, with North Africa and the Middle East being particularly low while across Europe it is higher (RITCHIE H and ROSER M, 2018).

Worldwide, alcohol consumption results in approximately 3 million deaths (5.3% of all deaths) annually. One of the major problems related to alcohol consumption is the loss of control over its intake, in which some cases become excessive drinkers and may eventually develop an Alcohol Use Disorder (AUD). AUD is characterized by compulsive alcohol use and loss of control over its intake, impairing not only executive skills, but also cognitive skills such as emotion regulation, working memory, attention and motivation (MEINHARDT MW, et al., 2021).

Ethyl alcohol is a short-chain hydrocarbon C2H5OH, has a chemical structure that has a polar part at one end and a non-polar part at the other, therefore being soluble in water and lipids, for example. This ambiguity in its polarity facilitates a more accelerated diffusion of ethanol through biological membranes, facilitating its absorption. In addition, it can target structures that have cell membrane bilayers, monolayers organelle (mitochondria or endoplasmic reticulum), and interact with membrane proteins in a systemic way (TESCHKE R, 2018).

As any multifactorial problem, the mechanism of alcoholism is not fully understood, the effects caused by alcohol abuse are known to depend mainly on behavior, biochemical homeostasis, health status, sex, body mass, age and genetic. Therefore, chronic ingestion of this substance can lead to multisystem diseases that can cause breast cancer, colon cancer, pancreatic disease, liver cirrhosis, diabetes, osteoporosis, arthritis, kidney disease, gastrointestinal disorder, immune system dysfunction, hypertension, coronary artery disease, alcohol-induced cardiomyopathy, heart failure, and psychiatry disorders (DGUZEH U, et al., 2018).

Although there are approved pharmacological treatments for the treatment of alcohol addiction, all have limited efficacy, and low prescribing rates, opening doors for the development of drug innovations and new treatment methodologies (HEILIG M, et al., 2019). For this reason, knowing the biological substrates related to alcohol and its effects is important because it allows us to understand (i) the evolution of the condition, that is, the allostatic point that would define the border between drug use and dependence, (ii) therapeutic targets and (iii) diagnostic markers. In physiological conditions, biochemical pathways are in balance (homeostatic point), preserving the systemic functions of the body organs, even after acute insults. Conversely, the chronical insult (as alcohol abuse) alters the substrate availability, determining a new energy demand in cell (allostasis) which also drives the effects on brain neurotransmission and mental states, at least in part (KOOB GF and COLRAIN IM, 2020; KOOB GF and LE MOAL M, 2001; GANZEL BL, et al., 2010; STERLING P, 2012).

Enzyme acetyl-CoA synthetase (E.C. 6.2.1.1, ACSS2) catalyzes acetyl-CoA formation from acetate with adenosine triphosphate (ATP) and CoA consumption and generates adenosine monophosphate (AMP) and pyrophosphate PP (EISENBERG M, 1995). ACSS2 also forms acetyl adenylate, which is a precursor of AMP and adenosine. Furthermore, the enzyme affects energy production, gluconeogenesis, acetate regulation, propionate, acetyl carnitine, immune system modulation, epigenetic, glutamate and gamma-aminobutyric acid synthesis and other pathways of cellular metabolism (GAO X, et al., 2016).

It is noteworthy that these pathways are influenced by alcohol and that the mRNA responsible for encoding the ACSS2 enzyme and its function can be affected by alcohol intake in a free-choice mice model. Only in the compulsive-like drinker mice exhibited an increased Acss2 mRNA levels in amygdala nuclei region of the brain (RIBEIRO AF, et al., 2017).

However, the exact mechanism underlying this health problem is unclear and for this reason, the aim of the study was to perform a systematic review with meta-analysis to better understand the effects of alcohol on the ACSS2 pathway.

METHODS

Procedures

A search was carried out in the electronic databases of Medical Literature Analysis and Retrieval System Online (*Medline*), *Biblioteca Virtual em Saúde*, and *Scopus*, using a combination of keywords including: *acetate* AND "*acetyl CoA* OR *acetyl coenzyme A* OR *acetyl phosphate* OR *acid propanoic* OR *acyl CoA short chain* OR *acyl coenzyme A short chain* OR *propionyl coenzyme A* OR *propionate OR propionic acid* OR *propionyl CoA* OR *propionyl coenzyme A*" AND "*alcoholism* OR *ethanol* OR *ethyl alcohol* OR *ethylic alcohol*". To manage the references, JabRef v. 4.3.1 and EndNote™ online were used. A systematic review, registered on PROSPERO (CRD42022310815).

Inclusion criteria

In order to find as much information as possible in the literature and taking into account the lack of studies related to the topic, studies published until December 2019 were included in this work. Among the correlations, the following stand out: (i) alcoholic humans and/or alcohol abuse; (ii) animal subjects exposed to alcohol; and (iii*) in vitro* studies on the effects of alcohol. The accepted languages were English and Portuguese.

Exclusion criteria

Some studies were excluded for having (i) no relation with the effects of alcohol, alcohol abuse, and/or dependence; (ii) no relation with the hypothesis presented in this study; (iii) results influenced by some type of treatment; (iv) inadequate statistical analyses; (v) duplicate data from the same author; (vi) duplicates; and (vii) no original data.

Selection of studies

A systematic review was conducted blindly by three reviewers. In case of disagreement between the results, the inclusion decision was achieved by consensus among the reviewers. The studies included in this analysis were in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) and Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

Statistical analysis

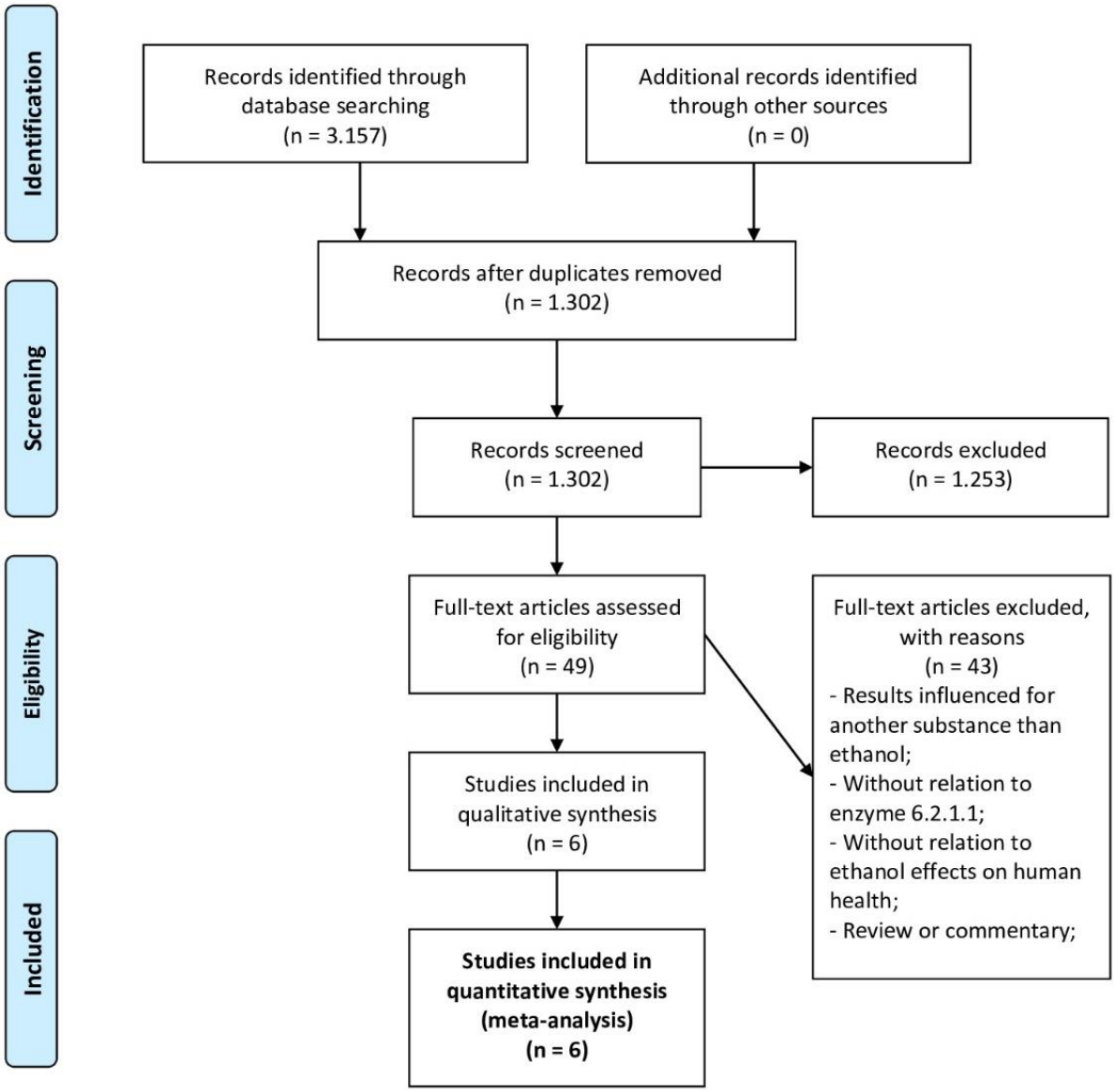
The data were tabulated in an Excel® spreadsheet and later analyzed in Review Manager v. 5.3. The variables considered were the levels of acetate, acetoacetate, acetyl-CoA, ATP, AMP, adenosine diphosphate (ADP), inorganic phosphate (Pi), and ACSS2 activity. Intergroup heterogeneity was estimated using the Higgins test (I^2), and the results were interpreted according to the Cochrane Handbook for Systematic Reviews of Interventions. The standardized mean difference and the weighted average of the meta-analysis were compared using the random model. The confidence interval (CI) was 95% and the significant difference was at $p < 0.05$. To verify the bias a funnel plot was used, and to estimate the main results a forest plot was used.

RESULTS

A total of 3,157 studies were identified in the databases, of which 1,302 were retained after deleting duplicates. Of these, 1,253 were not related to the proposed objectives. After this step, 49 studies remained eligible for full text reading. In the final screening stage, six studies were included for the meta-analysis (DAWSON AG and SMITH MM, 1986; FELLENIUS E, et al., 1972; FORSANDER OA and LINDROS KO, 1967; KISELEVSKI Y, 2003; LUKIVSKAYA OYA and BUKO VU, 1993; ZIMATKIN SM, et al., 2011) (**Figure 1)** (**Table 1**).

All studies included in this systematic review with meta-analysis had rats as a biological model, 49 (21 albino rats and 28 *Wistar* rats) in the control group and 53 (21 albino rats and 32 *Wistar* rats) in the experimental group (treated with ethanol). The control group consisted of 34 (15 albino rats and 19 *Wistar* rats) male rats and 15 (6 albino rats and 9 Wistar rats) female rats, while the treatment group consisted of 34 (15 albino rats and 19 *Wistar* rats) male rats and 19 (6 albino rats and 13 *Wistar* rats) female rats. Regarding the body weight of the rats and the dosage used in the experiments, rats weighing between 100 and 300g and two doses between 1.6g of ethanol/kg and 3.5g of ethanol/kg were used (**Table 1**).

Figure 1 – PRISMA Systematic flow diagram of study selection.



Source: Souza-Silva G, et al., 2022 ; based in Moher D, et al., 2009.

Table 1 - Compiled information about selected papers for inclusion.

Item	Study design	Sample size Group (n)	Outcome measures	Statistical methods	Experimental animals	Experimental procedures
Dawson AG and Smith KTS (1986)	The levels of metabolites were measured in the livers of rats treated with ethanol.	Control (9) | Ethanol (13)	Level of ethanol, acetaldehyde, acetate, acetoacetate, ATP, ADP, and Pi in the liver.	Comparing between control and ethanol groups.	Female Wistar rats weighing 150g–300g. The animals received food and water, ad libitum, throughout the treatment period.	Rats received a single i.p. injection of 0.154 M NaCl or 12.5% (w/v) ethanol in 0.154 M NaCl with a dose of 2.5g ethanol/kg body weight. The injections were given at the same time. Two hours after the injection the animals were euthanized by cervical dislocation and the liver was excised and then immersed in liquid nitrogen (the total elapsed time from cervical dislocation until liver freezing was less than 20sec). The measures were employed following the enzymatic method.
Fellenius E, et al. (1972)	The activity of the hepatic enzyme, acetyl-CoA synthetase, was measured in rats feeding on a liquid diet containing ethanol.	Control (6) | Ethanol (6)	Enzyme 6.2.1.1 activity.	Student's *t* test.	Male *Wistar* rats (Institute of Zoophysiology, Uppsala, Sweden). The rats were 40–76 days-old at the beginning of the treatment.	Rats were given a liquid diet (approximately 43% of carbohydrate, 40% of fat, and 17% of protein) without ethanol (control group) or with ethanol (ethanol group). After 10, 20, 30, 40, 50, 60, 70, and 80 days, rats were euthanized. The liver was quickly excised, and enzyme activity and total protein were quantified.
Forsander OA and Lindros KO (1967)	The level of acetyl-CoA was measured in the livers of rats treated with ethanol.	Control♂ (9) and Control♀ (6) | Ethanol♂ (9) and Ethanol♀ (6)	Level of acetyl-CoA.	No statistical test for comparison was employed.	Female and male albino rats (Alko, Helsinki, Finland) weighing 200–300g and fed a normal diet.	Rats received 1.6g of ethanol/kg body weight by gavage 1 hour before liver excision. Rats were anesthetized with Nembutal and then livers were excised and frozen. The acetyl-CoA level was determined by spectrophotometry.
Kiselevski Y, et al. (2003)	Rats classified in the medium-sleeping category were exposed to ethanol treatment. Various metabolites and the acetyl-CoA synthetase activity were measured in the brain.	Control (6) | Ethanol (6)	Level of acetate, acetyl-CoA, AMP+ADP | Enzyme 6.2.1.1 activity.	Student's *t* test.	Male *Wistar* rats weighing 100–120g. The rats were fed standard chow and housed in group cages in an air-conditioned room with a cycle of 12 hours of light / 12 hours of dark. The rats were divided into 3 groups according to the duration of ethanol-induced sleep time (after an acute test dose of ethanol 25% solution, 3.5 g/kg i.p.): long-sleeping (LS); medium-sleeping (MS); short sleeping (SS).	MS rats after 14 days of acute ethanol test were treated with a daily i.p. injection of 25% ethanol, 3.5g/kg of body weight. A control group received i.p. injections of 0.9% NaCl solution. The rats were euthanized by decapitation 24h after the seventh injection of ethanol or saline. The brains were removed at 0–4ºC, and the cerebral cortex was dissected. The enzyme activity was measured using the citrate synthetase method. The level of acetyl-CoA was measured by the enzymatic method. The level of acetate was determined by CG. The levels of ADP+AMP were measured by High Performance Liquid Chromatography (HPLC).

Item	Study design	Sample size Group (n)	Outcome measures	Statistical methods	Experimental animals	Experimental procedures
Lukivskaya OYA and Buko W (1993)	Rats were exposed to 40 days of ethanol treatment. The level of acetoacetate and the acetyl-CoA synthetase activity were determined in the liver and the brain.	Control (6) | Ethanol (6)	Level of acetoacetate | Enzyme 6.2.1.1 activity.	Student's t test.	Male albino rats weighing 140–180g. All rats received a standard laboratory diet and water (*ad libitum)* and were starved for 12 hours before they were euthanized.	Ethanol 25%v/v solution, 3g/kg body weight, was administrated by gavage for 40 days. Control rats were given the equivalent in distilled water, following a similar procedure. The rats were euthanized by decapitation 3h after the last dose of ethanol. The liver and brain were rapidly removed and cooled in liquid nitrogen.
Zimatkin SM, et al. (2011)	Rats classifying in the *Wistar* short-sleeping (wSS) and *Wistar* long-sleeping (wLS) categories were exposed to ethanol treatment. The metabolite acetate and acetyl-CoA levels were measured in the brain. The acetyl-CoA activity was measured in the brains of the rats classified as above as well as high alcohol sensitive (HAS) and low alcohol sensitive (LAS) rats.	Control (7) | Ethanol (7)	Level of acetate and acetyl-CoA | Enzyme 6.2.1.1 activity.	ANOVA one-way and *post hoc* using Scheffe test.	Male *Wistar* rats from the breeding colony of the Grodno State Medical University. Ninety rats were tested for the differences in sensitivity to the hypnotic effects of ethanol. The dose of 3.5 g/kg i.p. was administered and the duration of alcohol-induced sleep was measured by the interval from the loss of righting response to its recovery. The rats were divided into *Wistar* short-sleeping (wSS) and *Wistar* long-sleeping (wLS) groups.	Two weeks after the test of hypnotic effects of alcohol, seven rats from each group received ethanol solution 20% (3.5 g/kg) i.p., one hour before they were euthanized by decapitation. Another seven rats from each group received NaCl solution 0.85% i.p. After that the brain was quickly excised and the cortex dissected and frozen in liquid nitrogen. Brain samples from rats of the genetically selected lines LAS (low alcohol sensitive) and HAS (high alcohol sensitive) were utilized for the experiment.

Note: For all studies, all samples were included in the experiment; No randomization method was mentioned; No blinding was used.
Source: Souza-Silva G, et al., 2022.

Enzyme ACSS2 metabolites

The phosphorylated bases of adenosine (ATP, ADP e AMP) and Pi were increased 1.16 times by ethanol (CI 95%, 0.42, 2.10; $p = 0.003$). There was low heterogeneity among the studies ($I^2 = 8\%$; $Chi^2 = 2.18$, $p = 0.34$) (**Figure 2**) (**Table 2**). We found no asymmetry in the funnel plot, indicating no evidence of publication bias.

Table 2 - Phosphorylated adenosine and inorganic phosphate levels.

Study	Experimental			Control			Weight	Std. Mean Difference
	Mean	SD	Total	Mean	SD	Total		IV, Random, 95% CI
Dawson AG and Smith KTS (1986)[1]	1.70	0.1	4	1.59	0.2	4	31.50%	0.59 [-0.86, 2.03]
Dawson AG and Smith KTS (1986)[2]	5.39	0.3	4	4.99	0.4	4	27.80%	0.99 [-0.55, 2.54]
Kiselevski Y, et al. (2003)[3]	2.89	0.2	8	2.10	0.5	8	40.60%	1.97 [0.71, 3.12]
Total	**-**	**-**	**16**	**-**	**-**	**16**	**100%**	**1.26 [0.42, 2.10]**

Note: [1](ATP+ADP); [2](Pi); [3](AMP+ADP). Heterogeneity: $Tau^2 = 0.05$; $Chi^2 = 2.18$; $df = 2$ ($p = 0.34$); $I^2 = 8\%$. Teste of overall effect: $Z = 2.93$ ($p = 0.003$).
Source: Souza-Silva G, et al., 2022.

The levels of the acetate, acetoacetate, and acetyl-CoA metabolites increased 2.05 times by ethanol (CI 95%, 1.07, 3.04; $p < 0.0001$) (**Table 3**). We found no asymmetry in the funnel plot, indicating no evidence of publication bias.

Table 3 - Acetate, acetoacetate, and acetyl-CoA levels.

Study	Alcohol Group			Control Group			Weight	Std. Mean Difference
	Mean	SD	Total	Mean	SD	Total		IV, Random, 95% CI
Dawson AG and Smith KTS (1986)[1]	1.2	0.18	11	0.17	0.09	9	7.8%	6.65 [4.19, 9.11]
Dawson AG and Smith KTS (1986)[2]	0.04	0.01	3	0.04	0.01	4	11.2%	0.07 [-1.42, 1.57]
Forsander OA and Lindros KO (1967)[3]	32.7	12.6	6	18.2	5.1	6	11.9%	1.39 [0.07, 2.71]
Forsander OA and Lindros KO (1967)[4]	29.6	6.3	9	22.1	7.1	9	13.0%	1.06 [0.06, 2.07]
Kiselevski Y, et al. (2003)[1]	0.62	0.14	8	0.31	0.1	8	11.7%	2.41 [1.04, 3.78]
Kiselevski Y, et al. (2003)[4]	88.3	8.8	8	82.2	19.1	8	13.1%	0.39 [-0.60, 1.38]
Lukivskaya OYA and Buko W (1993)[2]	0.11	0.01	6	0.08	0.01	6	10.1%	2.81 [1.03, 4.59]
Zimatkin SM, et al. (2011)[1]	1	0.15	7	0.62	0.01	7	10.5%	2.96 [1.30, 4.62]
Zimatkin SM, et al. (2011)[4]	101.3	8.3	7	75.9	9	7	10.8%	2.75 [1.15, 4.34]
Total	**-**	**-**	**65**	**-**	**-**	**64**	**100%**	**2.05 [1.07, 3.04]**

Note: [1](acetate); [2](acetoacetate); [3](acetyl-CoA ♀); [4](acetyl-CoA ♂). Heterogeneity: $Tau^2 = 1.69$; $Chi^2 = 34.73$; $df = 8$ ($p < 0.0001$); $I^2 = 77\%$. Teste for overall effect: $Z = 4.07$ ($p < 0.0001$),
Source: Souza-Silva G, et al., 2022.

ACSS2 activity

The activity of the enzyme ACSS2 exhibited a significant, 2.07-fold, increase after alcohol treatment (CI 95%, 0.16, 3.97; $p = 0.03$) (**Table 4**). We found no asymmetry in the funnel plot, indicating no evidence of publication bias.

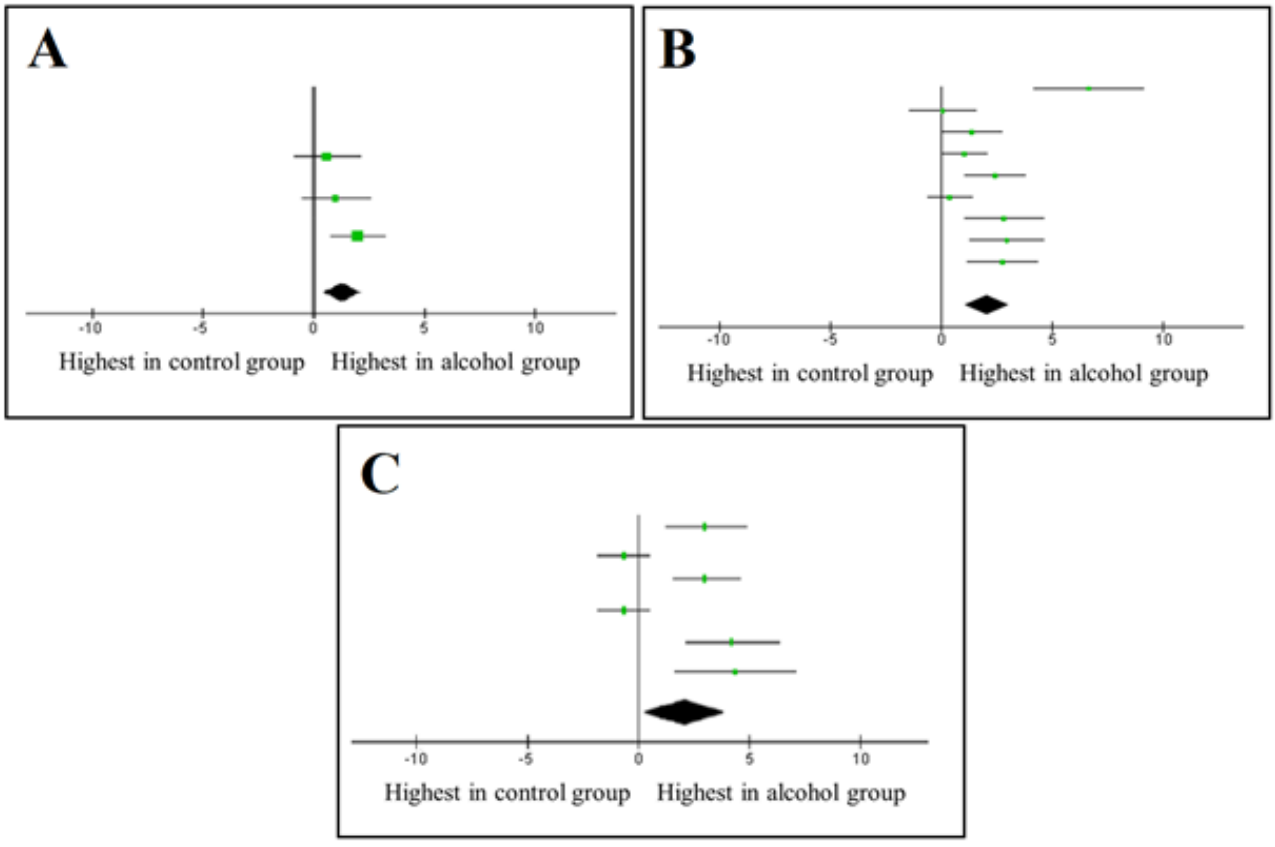
Table 4 - ACSS2 activity.

Study	Alcohol Group			Control Group			Weight	Std. Mean Difference
	Mean	SD	Total	Mean	SD	Total		IV, Random, 95% CI
Fellenius E, et al. (1972)[1]	7.5	1.08	6	4.5	0.69	6	16.5%	3.06 [1.18, 4.93]
Fellenius E, et al. (1972)[2]	5.3	1.35	6	6.1	0.88	6	18.3%	-0.65 [-1.82, 0.53]
Kiselevski Y, et al. (2003)[3]	3.48	0.3	8	2.56	0.27	8	17.3%	3.05 [1.49, 4.61]
Lukivskaya OYA and Buko W (1993)[3]	3.38	1.3	6	4.2	0.96	6	18.3%	-0.66 [-1.84, 0.51]
Zimatkin SM, et al. (2011)[3]	4.2	0.2	7	3.3	0.2	7	15.8%	4.21 [2.09, 6.33]
Zimatkin SM, et al. (2011)[4]	5.1	0.6	5	2.8	0.3	5	13.9%	4.38 [1.62, 7.14]
Total	**-**	**-**	**38**	**-**	**-**	**38**	**100%**	**2.07 [0.16, 3.97]**

Note: [1](liver; 20 days); [2](liver; 80 days); [3](brain; rats wSS and wLS); [4](brain; rats LAS and HAS). Heterogeneity: Tau^2 = 4.81; Chi^2 = 41.00; df = 5 (p < 0.00001); I^2 = 88%. Teste for overall effect: Z = 2.13 (p = 0.03).
Source: Souza-Silva G, et al., 2022.

There was low heterogeneity among levels of adenosine phosphorylate and inorganic phosphate (I^2 = 8%; Chi^2 = 2.18, p = 0.34). However, intergroup variability showed greater heterogeneity considering acetate, acetoacetate, and acetyl-CoA levels (I^2 = 77%; Chi^2 = 34.73, p < 0.0001), and ACSS2 activity (I^2 = 88%; Chi^2 = 41.00, p < 0.00001) (**Figure 2**).

Figure 2 - Forest plot on the comparison between the effects of alcohol on the control and self-consumption groups.



Legend: A - Effects of alcohol on different forms of adenosine phosphorylated and inorganic phosphate. **B** - Effects of alcohol on acetate, acetoacetate, and acetyl-CoA levels. **C** - Effect of alcohol on ACSS2 activity.
Source: Souza-Silva G, et al., 2022.

DISCUSSION

The meta-analysis showed an increase in the levels of phosphorylated bases of adenosine after acute treatment with alcohol, thus, indicating the susceptibility of adenosine metabolism to alcohol. For the acetylation metabolites, an increase in acetate and acetyl-CoA levels following acutely administrated alcohol was observed, but higher heterogeneity was also noted. Moreover, alcohol seems to have a biphasic effect on ACSS2 activity, depending on the duration of drug use. The increase or decrease in activity becomes more evident in short or long periods of alcohol use, respectively.

Dawson AG and Smith MM (1986) demonstrated that only one injection of alcohol (2.5g/kg) intraperitoneally (i.p.) in female *Wistar* rats altered the levels of metabolites in the hepatic tissue. Specifically, acetate and ADP increased, acetoacetate and Pi also increased but without statistical significance, while ATP was reduced (statistically nonsignificant). Kiselevski Y, et al. (2003) demonstrated that acute treatment with alcohol (3.5g/kg) i.p. in male *Wistar* rats increased levels of AMP and ADP, along with acetate and acetyl-CoA, however, without statistical significance for the last metabolite, in the brain cortex.

Forsander OA and Lindros KO (1967) also reported an increase in acetyl-CoA level in the liver of male and female rats after gavage administration of alcohol (1.6g/kg). Lukivskaya OYA and Buko VU (1993) found that alcohol (3g/kg), when administrated intragastrically for 40 days, increased acetoacetate levels in the livers of male albino rats. Zimatkin SM, et al. (2011) found an increase in the levels of acetyl-CoA and acetate in the frontal cortex of the brain of short-sleeping *Wistar* rats 1h after the injection of alcohol (3.5g/kg) i.p.

Alcohol promotes an increase in acetate, and this metabolite seems to potentiate the anesthetic effects of alcohol, and through an indirect effect, it also reduces the level of ATP and increases the level of AMP, the latter being acutely increased (CAMPISI P, et al., 1997; ZYDOWO MM, et al., 1993). Nagy LE, et al. (1990) and Fredholm BB and Wallman-Johansson A (1996) found that alcohol and acetate inhibit the recapture of adenosine via the nucleoside transporter, increasing its levels extracellularly. The adenosine A1 receptors are also required for astrocyte calcium activation to increase alcohol sedation (ERICKSON EK, et al., 2021).

Venkatraman A, et al. (2004) reported that the α subunit from ATP synthase (the adenosine binding site) in the inner mitochondrial membrane, and not the β subunit, was decreased in the livers of ethanol-fed rats. Adenosine plays a crucial role in energetic metabolism; however, alcohol has a negative impact on ATP synthesis and consequently on the intracellular process depending on mitochondrial oxidative phosphorylation (OXPHOS). Thus, even though alcohol provides the carbon to increase the energetic state of the cell, the excess of acetate would not be oxidized (in the presence of O_2), due to the reoxidation inefficiency of Nicotinamide Adenine Dinucleotide (NADH), compromising the efficacy of glycolysis and the citric acid cycle.

Recently, Barbier-Torres L, et al. (2022) found that mitochondrial organization, fatty acid β-oxidation, TCA cycle were altered in patient with alcoholic hepatitis. Supporting that glycolysis, gluconeogenesis, synthesis and degradation of ketone bodies, and pyruvate metabolism were impaired by alcohol. Moreover, OXPHOS respiratory complex I (NADH dehydrogenase), III (cytochrome b-c1) and V (ATP synthase complex) were reduced by alcohol, confirming that bioenergetic mitochondrial dysfunction is alcohol-induced.

Thus, alcohol use could contribute to a decoupling-like effect, as a result of shifting on entropy in the transition states of the enzymes in the metabolic process, which is mitochondrial-dependent. Moreover, it is important to note that acyl-CoA regulates the cellular energy phosphate status through an integration between glycolysis, gluconeogenesis and beta-oxidation pathways, by an inhibitory effect on glycolysis and stimulation of lipogenesis (SCHÖNFELD P and WOJTCZAK L, 2016).

Fifty-one days-old male Wistar rats, 51 days old, treated with alcohol for approximately 70 days, exhibited altered enzymatic activity in the liver (FELLENIUS E, et al., 1973). Almost after two weeks of alcohol exposure, ACSS2 activity increased. Then, after six weeks, reduced activity was observed, and it remained lower than in the control group. Lukivskaya OYA and Buko VU (1993) demonstrated that the treatment of male Wistar rats with alcohol (3g/kg) via intra-gastric gavage for 40 days decreased ACSS2 activity in the liver, heart, and brain (statistically nonsignificant for the last tissue). Kiselevski Y, et al. (2003) exposed male Wistar rats to

acute alcohol i.p. (3.5g/kg) and observed increased ACSS2 activity in the brain cortex. A similar result was obtained by Zimatkin SM, et al. (2011) through an analysis of the frontal cerebral cortex of short-sleeping (wSS) Wistar rats one hour after exposure to alcohol (3.5g/kg) i.p.

Fang T, et al. (2017) demonstrated that the hypnotic effects of alcohol are lower in C57BL/6Slac mice and adenosine A2A receptor knockout mice compared to the wild-type mice. In addition, CD73+/+ mice, with ecto-5'-nucleotidase activity, showed reduced adenosine A1 receptor activity and tolerance to the hypothermic and ataxic effects of acute ethanol, compared with the CD73-/- mice, which were ecto-5'-nucleotidase-deficient (ZHANG D, et al., 2016). ACSS2 activity was higher in the cerebral cortex of rats tolerant to the hypnotic effect of alcohol (wSS and low alcohol-sensitive rats) than intolerant rats (long sleeper [wLS] rats and alcohol-sensitive rats). Furthermore, the use of acetate instead of pyruvate for the formation of acetyl-CoA is more common in wSS than wLS mice (NAGY LE, et al., 1990).

Taken together, the results suggested that alcohol pharmacokinetics could contribute to better production of phosphorylated adenosine and acetyl-CoA in addition to propionate but only temporarily (e.g., with one drink), consisting of a brief and acute effect. In this way, alcohol could provide relief from the metabolic "noise," but insufficient maintenance of the metabolic homeostasis after alcohol abuse would become present in the organism. Impairing modulation in the kinetic of events dependent on these metabolites and messengers (acetate, acetoacetate, ADP, AMP, ATP) and ACSS2 activity would result in a new state of energy demand (allostasis), regulated by a switch between anabolic and catabolic processes, and even interfering with epigenetic control of gene expression (PIETROCOLA F, et al., 2015).

Moreover, it supports the theory of acute tolerance from LeBlanc AE, et al. (1975) and helps us understand the dissociation between alcohol-seeking behavior and the expression of behavioral sensitization in compulsive-like drinker mice, and in adolescent mice using a three-bottle choice procedure, or in humans (RIBEIRO A, et al., 2008; CARRARA-NASCIMENTO PF, et al., 2017; NONA CN, et al., 2018).

Recently, Wilson DF and Matschinsky FM (2020) reviewed subjects' alcohol metabolism and pointed to significant metabolic energy depletion as well as decreasing AMP-dependent intracellular signalization after the use of alcohol. Yet, these effects on metabolic processes contribute to alterations in neurotransmission (as glutamatergic, dopaminergic, GABAergic, etc.) and epigenetic change that drives the brain and mental states, which together could be a useful pathway to understand the neurobiological differences between alcoholic phenotypes, risk of organ damage, and syndromes and disorders associated with alcohol use (MEWS P, et al. 2019; EGERVARI G, et al. 2021; ERICKSON ET, et al., 2021).

Alcohol can alter ACSS2 kinetics and adenosine metabolite quantities over time, which provides a better understanding of the behavioral and biological transition related to alcohol use, dependence, and even relapse. With advances in neuroscience, with each passing day, there is more clarity about mechanisms and factors correlated to alcohol use. Thus, several pathways are better understood and may possibly serve as therapeutic targets for the treatment of alcoholism. For this, it is necessary to develop strategies for the detection of different states of behavioral transition. Besides, acetate differs between region of the human brain (thalamus, cerebellum, brainstem, and cortex) and this response could be associated with alcohol dependence (TANABE J, et al., 2021).

It is important to note that the studies included in this systematic review were not recent. This implies that the lack of evidence does not exclude the importance of acetyl-Coenzyme A synthetase pathway to promote a better understanding of alcohol effects.

However, it should be considered that the time of sample collection was not specified in the studies included in this meta-analysis. Furthermore, differences in sample processing time may have led to variations in the level of measured metabolites. And differences in the physiological levels of these metabolites may also have contributed to the heterogeneity observed in the meta-analysis. In addition, study limitations were: (i) a small number of studies were included in the meta-analysis; (ii) small sample size in each of the included studies; (iii) heterogeneous experimental procedures between studies; (iv) data heterogeneity; (v) the evidence reflects the effects of alcohol rather than the drug's mechanism of action itself; and (vi) a small number of negative results.

FINAL CONSIDERATIONS

This is the first systematic review with meta-analysis that simplifies and provides an initial basis on the effects of alcohol on the metabolism of the acetyl-Coenzyme A synthetase pathway. Although more studies are needed to understand the neurochemical processes related to ACSS2 activity, the data pointed to the vulnerability of its pathway under alcohol exposure. Moreover, alcohol had a supplement-like effect, increasing levels of phosphorylated adenosine, acetyl-CoA, acetoacetate, and acetate, at least for a short period of time. In addition to increasing ACSS2 activity, although this effect was also time-dependent.

ACKNOWLEDGMENTS

We thank the employees of the Pontifícia Universidade Católica de Minas Gerais for their assistance and infrastructure. The fellowships were granted from Pontifícia Universidade Católica de Minas Gerais, FIP-2017-268-1S.

REFERENCES

1. BARBIER-TORRES L, et al. Depletion of mitochondrial methionine adenosyltransferase α1 triggers mitochondrial dysfunction in alcohol-associated liver disease. Nat. Commun., 2022; 13(1): 557.
2. CAMPISI P et al. Role of adenosine in the ethanol-induced potentiation of the effects of general anesthetics in rat. European Journal of Pharmacology, 1997; 325: 165-172.
3. CARRARA-NASCIMENTO PF, et al. Ethanol Sensitization during Adolescence or Adulthood Induces Different Patterns of Ethanol Consumption without Affecting Ethanol Metabolism. Front. Behav. Neurosci., 2017; 11: 46.
4. DAWSON AG, SMITH MM. Increased ketogenesis in hyperthyroid rats metabolizing ethanol. Biochemical Pharmacology, 1986; 35(4): 596-574.
5. DGUZEH U, et al. Alcoholism: A Multi-Systemic Cellular Insult to Organs. International Journal of Environmental Research and Public Health, 2018; 15(6): 1083.
6. EGERVARI G, et al. Alcohol and the brain: from genes to circuits. Trends Neurosci., 2021; 44(12): 1004-1015.
7. EISENBERG M. The acetate-activating enzyme of Rhodospirillum rubrum. Biochim Biophys Acta, 1955; 16(1): 58–65.
8. ERICKSON EK, et al. Cortical astrocytes regulate ethanol consumption and intoxication in mice. Neuropsychopharmacology, 2021; 46(3): 500-508.
9. FANG T, et al. Adenosine A2A receptor mediates hypnotic effects of ethanol in mice. Scientific Reports, 2017; 7: 12678.
10. FELLENIUS E, et al. Changes in the activity of citrate lyase, malic enzyme and acetyl -CoA synthetase in rat liver after short-term and long -term feeding with ethanol. Br. F. Nutr., 1973; 29: 307-316.
11. FREDHOLM BB, WALLMAN-JOHANSSON A. Effects of ethanol and acetate on adenosine production in rat hippocampal slices. Pharmacology and Toxicology, 1996; 79: 120-123.
12. FORSANDER OA, LINDROS KO. Influence of ethanol on the acetyl-coenzyme A level of intact rat liver. Acta Chem. Scand., 1967; 21(9): 2568.
13. GANZEL BL, et al. Allostasis and the human brain: Integrating models of stress from the social and life sciences. Psychol Rev, 2010; 117: 134-174.
14. GAO X, et al. Acetate functions as epigenetic metabolite to promote lipid synthesis under hypoxia. Nature Communications, 2016; 7: 11960.
15. KISELEVSKI Y, et al. Acetate metabolism in brain mechanisms of adaptation to ethanol. Med Sci. Monit., 2003; 9(5): BR178-82.
16. KOOB GF, COLRAIN IM. Alcohol use disorder and sleep disturbances: a feed-forward allostatic framework. Neuropsychopharmacol, 2020; 45, 141–165.
17. KOOB GF, LE MOAL M. Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology, 2001; 24(2): 97-129.
18. LEBLANC AE, et al. Acute tolerance to ethanol in the rat. Psychopharmacology, 1975; 41(1): 43-46.
19. LUKIVSKAYA OYA, BUKO VU. Utilization of ketone bodies by the rat liver, brain and heart in chronic alcohol intoxication. Alcohol & Alcoholism, 1993; 28(4): 431-436.
20. HEILIG M, et al. Sommer, Developing neuroscience-based treatments for alcohol addiction: A matter of choice?. Transl. Psychiatry, 2019; 9, 255.
21. MEINHARDT MW, et al. Psilocybin targets a common molecular mechanism for cognitive impairment and increased craving in alcoholism. Sci. Adv., 2021; 7(47): eabh2399.
22. MEWS P, et al. Alcohol metabolism contributes to brain histone acetylation. Nature, 2019; 574, 717–721.

23. MOHER D, et al. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med, 2009; 6(7): e1000097.
24. NAGY LE, et al. Ethanol increases extracellular adenosine by inhibiting adenosine uptake via the nucleoside transporter. The Journal of Biological Chemistry, 1990; 265(4): 1946-1951.
25. NONA CN, et al. Behavioural sensitization to alcohol: Bridging the gap between preclinical research and human models. Pharmacology Biochemistry and Behavior, 2018; 173: 15-26.
26. PIETROCOLA F, et al. Acetyl Coenzyme A: a central metabolite and second Messenger. Cell Metabolism Review, 2015; 21(2): 805-821.
27. RIBEIRO AF, et al. Possible involvement of ACSS2 gene in alcoholism. J. Neural Transm., 2017; 124: 1151.
28. RIBEIRO AF, et al. Lack of relation between drug-seeking behavior in an addiction model and the expression of behavioral sensitization in response to ethanol challenge in mice. J Neural Transm, 2008; 115: 43–54.
29. RITCHIE H, ROSER M. Alcohol Consumption. 2018. Retrieved from: https://ourworldindata.org/alcohol-consumption. Accessed on: February 17, 2022.
30. SCHÖNFELD P, WOJTCZAK L. Short- and medium-chain fatty acids in energy metabolism: the cellular perspective. J Lipid res, 2016; 57(6): 943-954.
31. STERLING, P. Allostasis: a model of predictive regulation. Physiol Behav, 2012; 106: 5-15.
32. TANABE J, et al. Effects of acetate on cerebral blood flow, systemic inflammation, and behavior in alcohol use disorder. Alcohol Clin. Exp. Res., 2021; 45(5): 922-933.
33. TESCHKE R. Alcoholic Liver Disease: Alcohol Metabolism, Cascade of Molecular Mechanisms, Cellular Targets, and Clinical Aspects. Biomedicines, 2018; 6(4): 106.
34. VENKATRAMAN A, et al. Modification of the mitochondrial proteome in response to the stress of ethanol-dependent hepatotoxicity. The Journal of Biological Chemistry, 2004; 279(21): 22092-22101.
35. WILSON DF, MATSCHINSKY FM. Ethanol metabolism: The good, the bad, and the ugly. Medical Hypotheses, 2020; 140: 109638.
36. WORLD HEALTH ORGANIZATION (WHO). Global status report on alcohol and health 2018. 2018. Retrieved from: https://www.who.int/publications/i/item/9789241565639. Accessed on: February 17, 2022.
37. ZHANG D, et al. Ethanol tolerance affects endogenous adenosine signaling in mouse hippocampus. The Journal of Pharmacology and Experimental Therapeutics, 2016; 358: 31-38.
38. ZIMATKIN SM, et al. Acetate-dependent mechanisms of inborn tolerance to ethanol. Alcohol Alcohol, 2011; 46(3): 233-8.
39. ZYDOWO MM, et al. Acetate-Induced changes of adenine nucleotide levels in rat liver. Metabolism, 1993; 42(5): 644-648.