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Original Article

Zahedan Journal of Research in Medical Sciences

Journal homepage: www.zjrms.ir

The Effects of Acute and Chronic Exposure to Ethanol on Chicken Brain Homocysteine and Leptin

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Article information

Article history:
Received: 17 May 2013
Accepted: 19 June 2013
Available online: 28 Sep 2013
ZJRMS 2015 Feb; 17(2): 22-26

Keywords:
Ethanol
Homocysteine
Leptin
Embryonic stage
Chickens

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Abstract

***Background*:** Leptin is one of the possible mediators of ethanol intake. On the other hand, the concentration of total plasma homocysteine (Hcy) is a well-established indicator for the risk of cardiovascular disease and seems to be related to ethanol consumption. So, the aim of the present study was to investigate the effect of acute (70%) and chronic (10%) exposure to evaporated ethanol on: 1-brain leptin and Hcy concentration on the 15th day of embryonic development of chick. 2- brain leptin and Hcy concentration immediately after hatch of chick and 3- serum leptin concentration immediately after hatch of chick.

***Materials and Methods*:** In this experimental study 60 fertilized eggs were used. Eggs were divided into control; acute exposure to ethanol and chronic exposure to ethanol. Hcy was measured by using enzyme-linked assay and leptin was measured with the chick leptin radioimmunoassay kit.

***Results*:** Data showed brain Hcy concentration on the 15th day of embryonic stage of chicken that acute and chronic exposure to ethanol significantly ($p<0.05$) decreased, but did not have any effect on brain Hcy concentration immediately after hatch in chicken that acute and chronic exposure to ethanol during embryonic stages. Acute and chronic exposure to ethanol during embryonic stages significantly ($p<0.05$) increased brain leptin on the 15th day of embryonic stage, brain leptin immediately after hatch of chicken and plasma leptin immediately after hatch of chicken.

***Conclusion*:** Present results indicated that acute and chronic exposure to ethanol by evaporation in embryonic stage of chicken can change the brain Hcy, brain leptin and serum leptin.

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Introduction

Leptin is a 16 K-Da polypeptide hormone (167 amino acids). The OB gene codes for hormones are secreted mainly by adipose tissue, placenta, fetal tissue and membranes, and stomach. Leptin reaches the brain, crossing the blood-brain barrier or the chroroid-cerebrospinal fluid barrier and informs the brain about the size of the fat stores [1]. Leptin has a wide variety of central and peripheral actions such as reproduction, food intake, energy expenditure, lipid metabolism, immune system, blood pressure and angiogenesis [2, 3]. Recent evidence has shown leptin as a risk factor for vascular disease. It may be an important link between cardiovascular disease and obesity. Leptin has a procoagulnat, atherosclerotic role and platelet aggregation effect [4].

Homocysteine (Hcy) is a sulfur-containing amino acid produced from food methionine metabolism in the body. It can be converted to methionine or cysteine by remethylation or trans-sulforation cycles with some enzymes (MS, MTHFR, SAM) and cofactors (B6, B12). Folate as a methyl group donor is essential in the remethylation cycle, too. Hcy is transported from blood brain barrier into the CSF and brain, although human neural cells are capable of producing Hcy [5]. Insufficient folic acid, B6, B12 and impairment in enzymes functions cause hyperhomocysteinemia. Hcy>12 µM/dL has been shown as a risk factor for vascular disease, brain athrophy and neurodegenrative disease [6]. Hyper-homocysteinemia has been linked to atherosclerosis and thrombosis [7].

Ethanol has weakly charged molecules that move easily through the cell membrane, rapidly equilibrating between blood and tissue. Alcohol, at low doses can have some beneficial effects such as decreased rates of myocardial infarction, stroke, gallstone, and possibly vascular or Alzheimer's dementia, but the consumption of more than 2 standard drinks per day increases the risk for health problems in many organ systems and hormonal changes [8]. Ethanol intake by changes in adipose tissue and BMI can affect leptin concentration [9]. Some studies have indicated an inverse relation between alcohol use and leptin level [10]. In other investigations ethanol is shown as a powerful inducer of hyperleptinemia in both animals and humans. Alcohol intake has the potential to alter body weight as it is energy-dense and may also alter eating behavior at higher levels of consumption. Each of these

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lifestyle factors, therefore, has the ability to alter adipose tissue mass, possibly via leptin [9]. On the other hand, short-term and chronic ethanol intake influences Hcy concentration by changes in the methylation pathway, folate and cofactors concentration [11]. Ethanol-induced increase in serum Hcy levels has been observed in active alcoholics. Exogenous ethanol caused elevated endogenous brain Hcy level, reduced S-adenosyl methionine (SAM) levels, and increased S- adenosyl Hcy (SAH) levels, which correlated to increased brain caspase-3 activities [12].

The reported sequences of leptin from human, cow, pig, sheep, mouse, rat, dog, and chicken showed a high degree of sequence conservation. This similarity suggests a common function or mechanism of hormone across species [13]. On the other hand, in previous studies exogenous ethanol caused a 1.6 fold increase in chick brain Hcy level at 11 days of development [12].

The present study was to investigate the effect of acute (70%) and chronic (10%) exposure to evaporated ethanol on brain leptin and Hcy concentration on the 15th day of embryonic development of chick, brain and serum leptin, and Hcy concentration immediately after hatch of chick. Fifteenth day that we used in present study was according to our previous study [14].

Materials and Methods

In this experimental study all procedures were approved by the animal care committee of the Iran academy of veterinary medicine. This study was fundamental experimental. Sixty fertile, pathogen-free, cub eggs were purchased from Fars Poultry center. More than 20 eggs (natural fertility is rarely 100%) of each group (acute, chronic exposure, control) were incubated under standard conditions (75% humidity at 37ºC). Humidity and temperature were checked by thermometer and a hygrometer. Incubator turning (3 times a day) was considered essential in the early stages. For the last 3 days of incubation when the chick was preparing to hatch, turning was stopped.

Test groups: eggs were divided into three groups 1- control group (eggs incubated in normal condition, N=20), 2- chronic group (eggs incubated while the water in incubator was replaced by 10 % ethanol, N=20), and 3- acute group (eggs incubated while the water in incubator was replaced by 70 % ethanol at the 6th, 13th and 20th days). In all groups half of the eggs were examined on the 15th day of developmental stage and the other half were examined immediately after hatching of chick.

For the extraction of brain, single whole brain was mixed with a phosphate buffer solution after homogenization, the mixture was centrifuged, and the brain extract was prepared for measurement of brain HCY and leptin. HCY were assayed by liquid stable 2-part HCY reagent cobas mira plus and leptin was assayed by chicken leptin ELISA kit from Cosabio Inc. The data were analyzed by SPSS-18 program and using one way ANOVA and Tukey as post hoc test. Significant level was considered to be $p<0.05$.

Results

Our data showed that brain Hcy concentration significantly ($p=0.01$) decreased on the 15th day of embryonic stage of chicken in acute and chronic groups relative to control group (Fig. 1). Figure 2 shows that acute and chronic exposure to ethanol had no significant effect on Hcy concentration on the first day of hatch.

Brain Hcy concentration on the 15th day of embryonic stage and the first day of hatch had not significant difference between acute and chronic groups.

Present data showed that following acute and chronic exposure to ethanol, brain leptin significantly ($p<0.001$) increased on the 15th day of embryonic stage of chick and on the first day of chick hatching (Fig. 3, 4). Also, serum leptin significantly ($p<0.01$) increased following acute and chronic exposure to ethanol on the first day of chick hatching (Fig. 5).

Brain leptin concentration on the 15th day of embryonic stage and the first day of hatch, significantly was lower in acute than chronic exposure to ethanol. But serum leptin concentration on the first day of hatch was higher in acute than chronic exposure to ethanol.

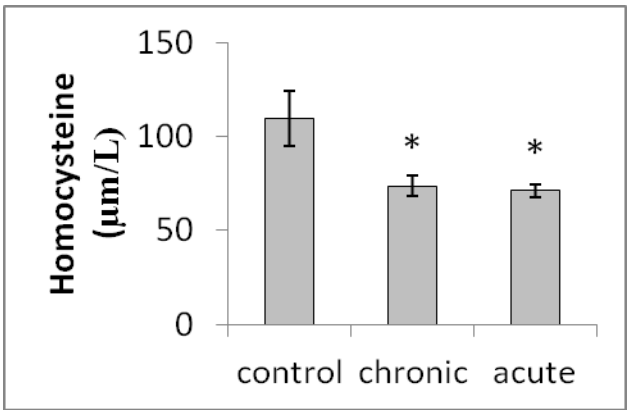

Figure 1. Effect of acute and chronic exposure to ethanol on brain Hcy on the 15th day of embryonic stage. *Significant difference relative to control ($p<0.05$)

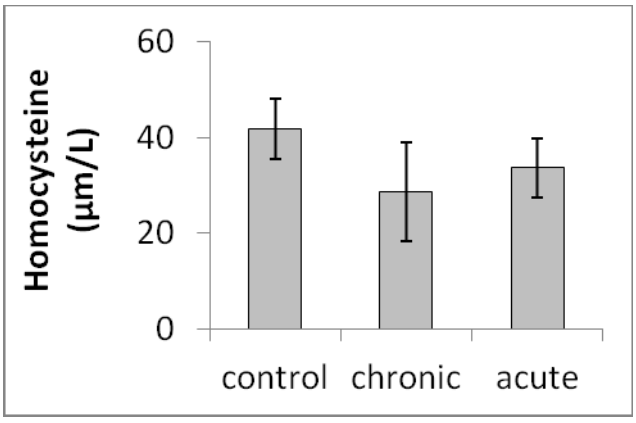

Figure 2. Effect of chronic and acute exposure to ethanol on brain Hcy on the first day of chick hatching

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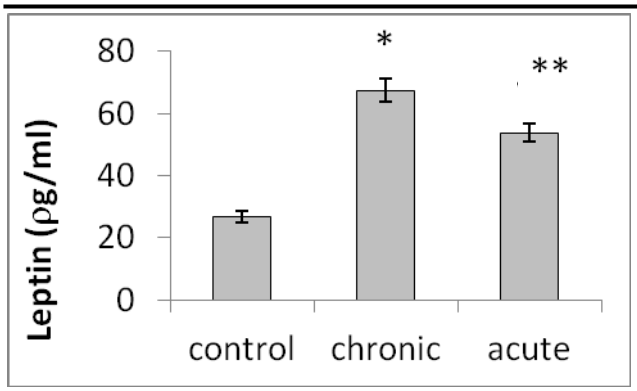

Figure 3. Effect of acute and chronic exposure to ethanol on brain leptin on the 15th day of embryonic stage
*Significant difference relative to control (p<0.05)
**Significant difference relative to chronic (p<0.05)

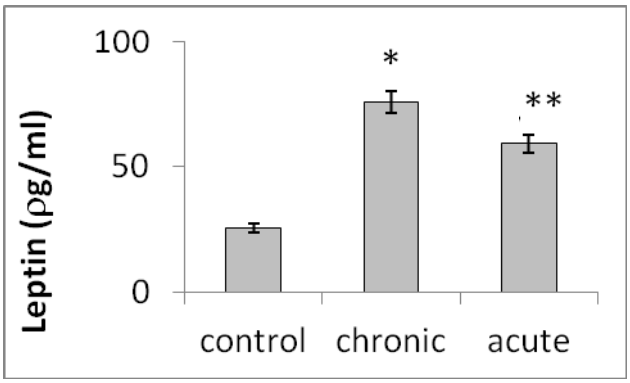

Figure 4. Effect of acute and chronic exposure to ethanol on brain leptin on the first day of chick hatching
*Significant difference relative to control (p<0.05)
** Significant difference relative to chronic (p<0.05)

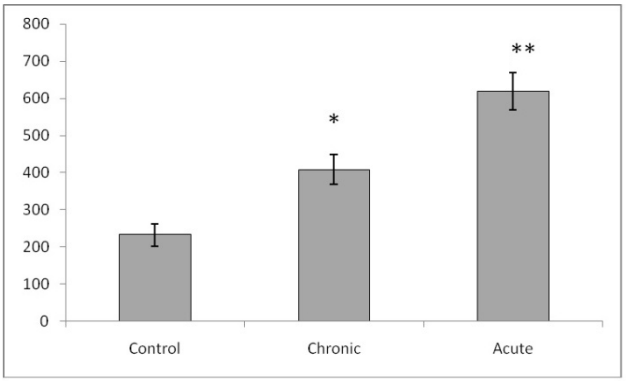

Figure 5. Effect of acute and chronic exposure to ethanol on serum leptin on the first day of chick hatching
* Significant difference relative to control (p<0.05)
** Significant difference relative to chronic (p<0.05)

Discussion

Leptin and Hcy as atherosclerotic and intima media thickness factors have great roles in almost all disease physiopathology [15].

In the present investigation ethanol in acute and chronic exposure causes a significant decrease in chick brain Hcy on 15th day of embryonic stage. Ethanol intake in different amounts can affect both of these pathogen factors. In some studies plasma Hcy was indicated as the blood alcoholization factor [16]. However, in other studies there was an inverse relation between Hcy and ethanol [17, 18].

It should be noted that an adequate amount of vitamin B groups and folate in egg help Hcy removal pathway and prevents brain Hcy level elevation in embryos. For instance, chicken egg naturally contains 22 µg of folate [12]. Furthermore, it is supposed that hepatic enzymes and methylation-transsulforation pathway are activated by ethanol, causing more Hcy removal. Also, during fertile eggs incubation period, embryos do not have methionine intake as a precursor of Hcy. Moreover, Lakashman et al. found that ethanol causes an increase in paraoxanase 1 (PON1) by upregulation of liver PON1 expression. PON is known as an antioxidant enzyme which can detoxify Hcy metabolite [19]. Also, ethanol in acute and chronic exposure to ethanol amounts causes a significant rise in S-adenosyl methionine and that activates methylation pathway in the hepatocytes, converting Hcy to methionine [11].

In the present study acute and chronic exposure to ethanol had no effect on one day chick brain Hcy. That could be a result of folate and vitamin B groups' shortage in the last embryonic days. Sakuta et al. showed a different relation between ethanol and plasma Hcy (elevating, decreasing, unchanged). In this study they pointed out that in hypofolatemia condition, the relation between ethanol and Hcy would be positive.

Lack of folate causes methylation cycle impairment. In addition, Belich et al. showed that, ethanol gradually decreases liver ability in folate maintenance and storage [20]. Bree et al. also explained an inverse relation between folate and Hcy level [21]. So that a 20% increase in folate level was followed by a 3.2% decrease in Hcy. This relation is more prominent in alcoholics (5.4%). Folate has a great role in methyl donation in remethylation cycle. In the final days of embrynoic stage, the lack of folate would be one reason for brain Hcy accumulation.

However, Shinohara et al. indicated that ethanol has different effects in different species, for instance, mice ethanol showed hyperhomocysteinemia, whereas rats showed no changes in Hcy level [22].

In the present study acute and chronic exposure to ethanol lead to an increase in the concentration of leptin on 15th day of chick brain embryo, and acute and chronic exposure to ethanol lead to an increase in the concentration of leptin in both of the brain and serum on the first day of hatching in chick. It is supposed that the leptin elevation was due to the ethanol effects on weight gain and adiposity. Short and long chain leptin receptors were seen on the 5th and 17th day of the developmental stage in liver, adipose tissue and yolk sac [13]. Leptin regulates energy balance and expenditure in embryonic period. Donahue et al. also confirmed the positive relation between adiposity and leptin level [10]. Balasubramaniyan et al. pointed out the influential effects of ethanol on cytokine synthesis in hepatocyts [23]. Cytokines, particularly TNF-α, are one of the most

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important stimulating factors for leptin elevation. In addition, ethanol causes a hypothalamic resistance to leptin and the level of this polypeptide begins to rise. Moreover, Donahue et al. showed that ethanol as a high energy molecule can have an effect on glucose and insulin plasma level. Insulin resistance increases leptin level in plasma and brain [10]. In the present study on the first day of hatch brain leptin was higher in the chronic than acute exposure to ethanol, but serum leptin was higher in the acute than chronic exposure to ethanol.

It seems that the enzymes for synthesis and metabolism of leptin are different in brain and serum, so the effect of acute and chronic ethanol in brain and serum leptin concentration was different. Patterson-Buckendahl et al. reported that chronic ethanol had less effect than acute ethanol on serum leptin [24].

In the present study: 1. Acute and chronic exposure to ethanol leads to decline of brain Hcy concentration on the 15th day of embryonic stage and the first day of hatch. There is no difference between acute and chronic exposure to ethanol. 2. Acute and chronic exposure to ethanol leads to increase of brain leptin concentration on the 15th day of embryonic stage and the first day of hatch and serum leptin on the first day of hatch. There is no difference between acute and chronic exposure to ethanol. Brain leptin was higher in chronic than acute exposure to ethanol on the 15th day of embryonic stage and the first day of hatch, but serum leptin was higher in acute than chronic exposure to ethanol on the first day of hatch.

According to our results it seems that cardiovascular disorder that caused by alcohol consumption, may be due to change in Hcy and leptin.

Acknowledgements

We want to thank Datis Teb company and Dr Saadat's laboratory for their excellent technical support.

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

All the authors can confirm that there is no financial or other relationship that would cause a conflict of interest.

Funding/Support

This paper had been done by Shiraz University.

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***Please cite this article as*:** Taherianfard M, Nazifi S, Farahani Z. Acute and chronic exposure to ethanol in developmental stage and chicken brain homocysteine and leptin. Zahedan J Res Med Sci. 2015; 17(2): 22-26.

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