DOI: http://dx.doi.org/10.1590/1413-785220192706222834

CHRONIC CONSUMPTION OF ALCOHOL ADVERSELY AFFECTS THE BONE OF YOUNG RATS

CONSUMO CRÔNICO DE ÁLCOOL AFETA NEGATIVAMENTE O OSSO DE RATOS JOVENS

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ABSTRACT

Objective: To assess the effect of chronic alcohol consumption on the longitudinal growth of the tibia and bone quality parameters in young rats under an experimental setup. METHODS: The control (n=10) rats received only water. The ethanol (n=10) rats received ethyl alcohol at concentrations established in the protocol for the induction of chronic alcohol consumption. The blood samples were immediately collected via cardiac puncture and processed to evaluate the levels of alkaline phosphatase by automated spectrophotometry. Following blood sample collection, both tibias were dissected, and weighed; the tibial length was measured., and the samples were stored in a freezer for future analysis of the bone mineral content and mechanical resistance, known as maximal load and stiffness. RESULTS: Compromised bone health, with a 35.3% decrease in the serum alkaline phosphatase levels ($p < 0.01$), a 10% decrease in the tibial mass ($p < 0.05$), and a 5.3% decrease in the tibial length ($p < 0.0001$) were noted. Furthermore, a 10% decrease in the bone mineral density was observed ($p < 0.01$), which led to a 17.2% decrease in the maximum strength ($p < 0.01$) and 22.6% decrease in stiffness ($p < 0.001$). CONCLUSION: Chronic consumption of alcohol affected the bones of young rats, making them weaker and osteopenic. In addition, the long bones were shorter, suggesting interference with growth. ***Level of Evidence III, Case Control Study.***

Keywords: Ethanol. Bone development. Bone density. Tibia. Rats.

RESUMO

Objetivo: Verificar a influência do consumo experimental crônico de álcool no crescimento longitudinal da tíbia e em parâmetros de qualidade óssea de ratos jovens. Métodos: Dez ratos controle receberam água, outros dez receberam álcool etílico nas concentrações estabelecidas no protocolo para indução. Após eutanásia, as amostras de sangue foram coletadas por punção cardíaca e processadas para avaliar os níveis de fosfatase alcalina por espectrofotometria automatizada. Após a coleta de sangue, ambas as tíbias foram dissecadas, pesadas e medidas em comprimento. Foram realizadas análises do conteúdo mineral ósseo e resistência mecânica, por meio da análise da força máxima e rigidez. Resultados: Houve comprometimento da saúde óssea, com redução de 35,3% no nível de fosfatase alcalina no plasma ($p<0,01$), redução de 10% na massa da tíbia ($p<0,05$) e queda de 5,3% no comprimento das tíbias ($p<0,0001$). Também foi observada redução de 10% na densidade mineral óssea ($p<0,01$), que levou à redução de 17,2% na força máxima ($p<0,01$) e 22,6% na rigidez ($p<0,001$). Conclusão: O consumo crônico de álcool afetou os ossos de ratos jovens, tornando-os mais fracos e osteopênicos. Ainda, os ossos longos eram mais curtos, sugerindo interferência no crescimento. ***Nível de evidência III, Estudo caso-controle.***

Descritores: *Etanol. Desenvolvimento Ósseo. Densidade Óssea. Tíbia. Ratos.*

Citation: Rosa RC, Rodrigues WF, Miguel CB, Cardoso FAG, Espindula AP, Oliveira CJF, Volpon FB. Chronic consumption of alcohol adversely affects the bones in young rats. Acta Ortop Bras. [online]. 2019;27(6):321-4. Available from URL: http://www.scielo.br/aob.

INTRODUCTION

Alcohol consumption is common throughout the world in different social and cultural contexts. Types of alcohol consumption differ between: only occasional consumption; heavy chronic alcohol consumption and binge drinking, being, at the moment, common among young people and adolescents.[1] The abusive consumption of alcohol can be harmful to different tissues and organs, including for example bones.[2]

To bone health, the excessive use of alcohol is concerned between young people, as it usually occurs when peak bone mass is reached.[3] Even in the case of this evidence, there is little information regarding the damage in the skeletal system of adolescents who make excessive use of alcohol[4], mostly the attainment of growth and peak bone mass.

All authors declare no potential conflict of interest related to this article.

The study was conducted at the Laboratory of Human, Universidade Federal do Triângulo Mineiro, Uberaba, MG, Brazil.
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Article received in 04/22/2019, approved in 09/10/2019.

Some research indicates that chronic alcohol use may interfere with bone metabolism and cause osteoporosis[2,5] by inhibiting osteoblastic cells.[6,7] Other authors believe that alcohol has a negative impact on mineral content, but does not interfere with bone growth.[8] Thus, the aim of the study was to evaluate the effect of experimental chronic alcohol consumption in growing rats on longitudinal growth of the tibia and parameters of bone quality.

MATERIAL AND METHODS

Experimental Design

According to well established methods, male Wistar rats (Rattus norvegicus albinus var. Wistar) were housed under standard laboratory conditions (room temperature 22 ± 2°C, humidity 55 ± 5%, 12 h light-dark cycles) with free access to tap water and chow (Nuvilab CR-1, Colombo, PR, Brazil).[9]

This study was carried out in strict accordance with international guidelines, as recommended in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The experimental protocol was approved by Ethical Animal Committee from the Federal University of Triângulo Mineiro - Brazil (CEUA/UFTM – Nº 323/2014). All euthanasia occurred with overdose of thiopental sodium injected intraperitoneally, and all efforts were made to minimize suffering.

The rats weighed 300 to 350 g (80–100 days old) and were kept in the laboratory environment during 1 week for acclimatization and were randomly distributed into two groups: Control (n=10) - rats received tap water ad libitum and Ethanol (n=10) – rats received 20% (v/v) ethanol in their drinking water.[10] To avoid loss of animals, the ethanol-treated group was submitted to a brief and gradual adaptation period. The animals received 5% ethanol in their drinking water in the 1st week, 10% in the 2nd week, and 20% on the 3rd and 4th week. The animals received 5% ethanol in their drinking water in the 1st week, 10% in the 2nd week, and 20% on the 3rd and 4th week. After this period, the experimental period started, with a concentration of 20% for eight weeks (2 months) until the end of the 12th week. All animals were housed in standard laboratory cages, with the same number of animals per box, allowing similar gait activity.

The animals were inspected daily and weighed weekly. After euthanasia, followed by Cardiac Puncture Blood Collection. Subsequently, both tibias were dissected, weighed, length measured and were stored in a freezer for future analysis of the bone mineral content and mechanical resistance: maximal load and stiffness. A planned euthanasia was performed

Analysis of alkaline phosphatase activity in blood plasma

After obtaining whole blood, the material was centrifuged at 1831g for 10 minutes and the serum was obtained. The determination of alkaline phosphatase was performed using an automated spectrophotometer (COBAS INTEGRA 400; Roche Diagnostics, São Paulo, SP, Brazil), following the manufacturer's instructions for commercial kits (Roche Diagnostics, São Paulo, SP), and all quality control recommendations for experimental analytical evaluation were performed.11 The results were expressed in UI/L that corresponds to 0.01667 μkat/L.

Bone length

The length of tibias was obtained with a digital caliper (Series 530, Mitutoyo, Suzano, SP, Brazil) by three consecutive measurements and average calculation.

Bone Mineral Density

The bone mineral density was determined by dual-energy X-ray absorptiometry (DXA) using a Lunar DPX-IQ densitometer (Lunar; software version 4.7e, GE Healthcare, Chalfont St. Giles, United Kingdom) with software for small samples. The tibias were immersed in ethanol in a small container and scanning of the entire bone was performed. Then, the region of interest was delimitated by a square measuring 0.90 cm^2 using the tibial tuberosity as an anatomical landmark.[12]

Mechanical Testing

The entire bone was tested in 3-point flexion. The bone extremities rested on two metallic supports that were 25 mm apart, and a progressive load was vertically applied at the center of the posterior surface of the bone at a constant displacement rate of 1 mm/min, until failure.[12] The testing device (EMIC, São José dos Pinhais, PR, Brazil) was equipped with a 500 N load cell, and the load-deflection curve was obtained in real time. The maximal load and stiffness were calculated by a specific software (TESC software, version 13.4, São José dos Pinhais, PR, Brazil).

Statistical Analysis

SPSS (SPSS for Windows - Version 11.0 - SPSS inc.,) was used for statistical analysis, and GraphPad Prism 5.0 was used for graphical presentation of the data. Data were initially submitted to descriptive analysis, with a calculation of means and standard deviations. Variables were tested by the Shapiro-Wilk test for normality and analysis of variance. For normal distributed variables, Student´s t parametric test was applied to compare the two groups. Differences were statistically significant at 5% reliability ($p < 0.05$).

RESULTS

The activity of alkaline phosphatase in blood plasma after 60 days of exposure to alcohol consumption was 35.3% lower in relation to the control group ($p < 0.01$). The mean values obtained in the control animals were 82.8 ± 20.7 IU/L and 53.6 ± 15.3 IU/L in the ethanol group (Figure 1A). The tibia mass of the ethanol group (0.78 ± 0.08 g) showed a significant reduction of 10% ($p < 0.05$), compared with the control group (0.70 ± 0.07 g) (Figure 1B). The mean tibia length from ethanol group was 5.3% shorter than in the control group ($p < 0.0001$). The tibias of control animals averaged 47.2 ± 1.0 mm, while the mean in the ethanol group was 44.7 ± 1.3 mm (Figure 1C). The bone mineral density of the tibias from the ethanol group was reduced by 30% ($p < 0.01$) compared with the control group. The average bone mineral density in the control group was 0.010 ± 0.002 g/cm^3 and in the ethanol group was 0.007 ± 0.002 g/cm^3 (Figure 1D). The maximal load of the tibias from ethanol group (82.5 ± 13.5 N) was reduced by 17.2% ($p < 0.01$), compared with the control group (99.7 ± 21.3 N) (Figure 2A). The mean stiffness of the tibias from ethanol groups was significantly decreased by 22.6% in comparison with the control animals ($p < 0.001$). The mean stiffness of the tibias of the control animals was 146.95 ± 31.20 N/mm and that of those in the ethanol group was 113.78 ± 25.18 N/mm (Figure. 2B).

DISCUSSION

Long-term alcohol consumption, besides being related to several behavioral pathologies, causes a multiplicity of biochemical, physiological and clinical abnormalities.[13,14] Heavy alcohol use has been associated with structural alterations in several tissues.[2,15]

Regarding bone health and osteoporosis, alcohol consumption is associated with reduced bone mass and increased risk of fracture.5,6 (5,6). In the literature, it is reported that young people between the ages of 18 and 30 (approximately 20% of women and 25% of men) participate in at least one episode of drinking every month, comprising 6 or more doses per occasion.[16] These data are in agreement with those of another study on young adult drinking behavior, which suggests that problem drinking behaviors that begin during adolescence

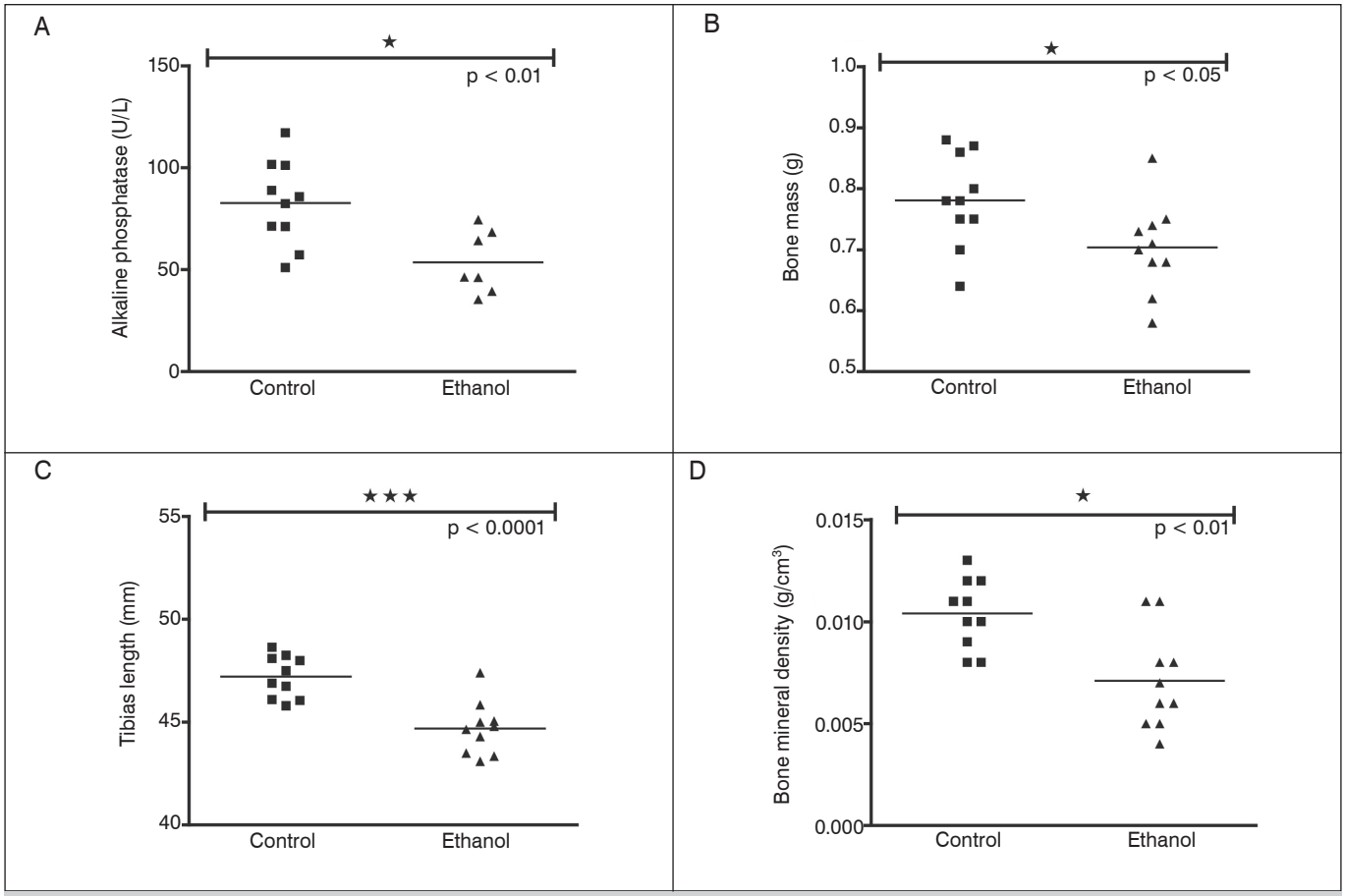

Figure 1. Biochemical analysis, skeletal development, and bone mineral content. A. alkaline phosphatase; B. bone mass; C. tibial length; D. bone mineral density. Statistical tests: Student´s t. $p < 0.05$. The asterisks indicate the comparison with significant difference.

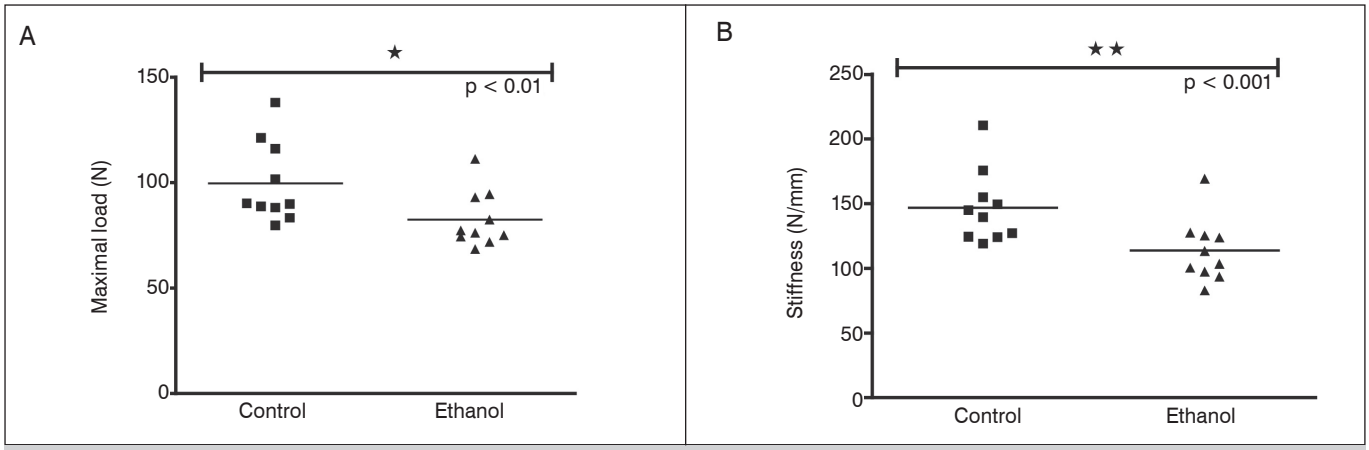

Figure 2. Analysis of mechanical resistance. A. maximal load; B. stiffness. Statistical tests: Student´s t. $p < 0.05$. The asterisks indicate the comparison with significant difference.

(ages 16–19) tend to continue into the early adult years (ages 30–31),[17] encompassing the most critical periods related to peak bone growth and accruement of bone mass.[3]

In the literature it has been described that alcohol can lead to compromises in the architecture of spongy bone, decrease bone mineral mass and inhibit bone growth in immature rats.[18] Evidence describes that a considerable proportion of adolescents and young adults use alcohol compulsively.[4] Our results demonstrate that exposure to experimental chronic consumption of alcohol significantly compromises bone health as demonstrated by the negative repercussions in bone mineral content and mechanical resistance. Additionally, the long bones were shorter, suggesting interference with growth.

In the current study, we used immature animals, making it possible to detect the detrimental effects on bone growth. These effects caused a reduction of 5.3% in tibia length, which may be related to the fact that chronic alcohol consumption seems to promote changes in bone metabolism due to nutritional deficiencies, liver damage, and hypogonadism. Thus, the etiology of alcohol-associated bone disease is multifactorial.

Excess alcohol increases urinary calcium, magnesium and zinc excretion. Zinc deficiency has been associated with osteoporosis caused by hypogonadism, which decreases the secretion of sex hormones. Moderate and prolonged alcohol consumption raises serum parathyroid hormone levels and may stimulate cortisol secretion. Chronic consumption of alcohol interferes in metabolism of vitamin D. These changes caused by alcohol consumption contribute to a reduction in bone formation, which results in osteopenia and increases the risk of fractures.[18]

As expected, the aforementioned characteristics are confirmed by the results of mechanical tests; tibias from exposed animals were weaker and less rigid. Stiffness is a parameter that represents how the bone deforms, and the maximal load refers to the bone mineral content and mechanical resistance. These results are consistent with some studies showing that alcohol ingested by immature rats provides inhibition of bone growth, decreased BMD which negatively impacts bone architecture.19 (19). These studies too teem that may not major loss of the cortical doma esponjoso has been associated to pesces adult after the status of the adult stereo, in the adult subordined status in the development study.[19]

Our current results show a significantly reduction of 30% in BMD and 22.6% in stiffness. The metabolic changes were expressed by a reduction of 35.3% in plasma concentrations of alkaline phosphatase for the animals from the ethanol group. Because the majority of serum alkaline phosphatase during the growing period is of skeletal origin,[18,19] these findings may also reflect a depression in bone metabolism. Thus, chronic alcohol consumption affected the bones of young rats, making them weaker and osteopenic. In addition, the long bones were shorter, suggesting interference in the growth.

ACKNOWLEDGEMENTS:

This research was supported by the Conselho Nacional de desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Fundação de Ensino e Pesquisa de Uberaba (FUNEPU) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). We appreciate the support of the following laboratory technicians: Rui Marcos da Silva, Rogério Lélis Rocha, Jonas Tadeu da Silva, Júlio César Abdanur and Marcelo do Carmo.

AUTHORS' CONTRIBUTIONS: Each author contributed individually and significantly to this study. RCR (0000-0003-3157-0826)*: Substantial contribution to the conception of the work, data interpretation, and critical review of the intellectual content. WFR (0000-0002-3426-2186)*: Analysis of microscope slides, writing of the manuscript, and statistical analysis of the data. CBM (0000-0002-1834-1394)*: Biochemical analysis, manuscript writing and manuscript revision. FAGC (0000-0001-7520-2879)*: Manuscript review and final approval of the version of the manuscript to be published. APE (0000-0002-9282-4482)*: Manuscript review and data interpretation. CJFO (0000-0003-2211-7333)*: Review of the manuscript and intellectual concept. JBV (0000-0002-2120-0138)*: Manuscript review, statistical analysis of the data, review of the intellectual concept, and development of the research project. *ORCID (Open Researcher and Contributor ID).

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