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The Effects of Chronic Binge Alcohol Consumption on the Development of the Femur of Adolescent Sprague Dawley Rats

Efectos del Consumo Excesivo Crónico de Alcohol en el Desarrollo del Fémur de Ratas Adolescentes Sprague Dawley

Ndabenzinhle Mngoma[1]; Diana Pillay[1,2] & Akaashni Bhika[1]

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SUMMARY: The impact of light, moderate, and heavy alcohol exposure on bone health has been investigated. However, there is a lack of literature concerning the effects of binge alcohol consumption on skeletal health in adolescents. Therefore, our study aimed to investigate how chronic binge alcohol consumption affects the development of femurs in adolescent Sprague Dawley rats. The study comprised of using seven-week-old Sprague Dawley rats (n = 12), randomly divided into two groups: the alcohol group, which received 20 % alcohol at 3g/kg body weight, and the pair-fed control group, which received an isocaloric equivalent of maltose dextrin through oral gavage for four weeks (administered three days a week on alternate days). To assess trabeculae in both the proximal and distal epiphysis and measure the femur's cortical dimensions, we employed three-dimensional micro-Focus X-ray computed tomography (3D-mCT) and volume graphics studio® software. Additionally, a three-point bending test was performed to examine the effects of alcohol on bone strength. The results indicated disturbances in the trabecular morphometry of the proximal epiphysis in rats exposed to alcohol. However, the trabecular morphometry in the distal epiphysis remained unaffected. Osteometric measurements did not show significant changes due to chronic binge alcohol consumption, and the tensile strength of the bones was also unaffected. These findings suggest that chronic binge alcohol consumption may have detrimental effects specifically on the trabeculae in the proximal epiphysis.

KEY WORDS: Adolescent femur; Chronic binge alcohol; Epiphysis; Tensile strength; Trabecular.

INTRODUCTION

Excessive alcohol consumption is known to have a range of detrimental effects on public health, including alcohol-related accidents, violence, and crime, and may disturb the integrity of bone development and the internal architecture of the osseous tissue (Reed *et al*., 2002; Morojele & Ramsoomar, 2016; Pillay & Ndou, 2021). South Africa is known to have a high prevalence of dangerous drinking practices, such as binge drinking (Morojele & Ramsoomar, 2016). This is a major public health concern as high levels of binge drinking are observed in the adolescent population, globally (Morojele & Ramsoomar, 2016). Binge alcohol drinking is defined as having had five or more drinks of alcohol in a single drinking episode (National Institute on Alcohol Abuse and Alcoholism, 2004; Föger-Samwald *et al*., 2018). The adolescent period is critical in the growth of bone and attaining peak bone mass (Das *et al*., 2017). Hence, alcohol consumption during this period may cause interruptions in bone growth and development (Lauing *et al*., 2008). Alcohol is known to decrease peak bone mass causing an increased risk of fractures and the early onset of osteoporosis (Sampson *et al*., 1996).

Previous studies have reported that chronic alcohol consumption may have a negative impact on bone growth and development resulting in osteoporosis later in life by inhibiting osteoblastic cells (Mikosch, 2014). However, the mechanism involved in this is not elucidated which needs further investigation. Also, Sampson in 1998 reported that alcohol had an adverse impact on bone mineral content but did not interfere with bone growth (Sampson, 1998). However, a study by Rosa *et al*. (2019) found that bone growth was reduced in alcohol-exposed rats.

Although it is known that alcohol perturbs the osseous tissue there is limited research regarding the damage to the skeletal system of adolescents who consume chronic alcohol consumption in a binge pattern.

[1] School of Anatomical Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.
[2] Department of Human Anatomy and Histology, School of Medicine, Sefako Makgatho Health Sciences University, Ga-Rankuwa, South Africa.
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Thus, the current study sought to evaluate the effect of chronic binge alcohol consumption in growing rats on the longitudinal growth of the femur and tensile strength of the bone. To achieve this, the current study used three-dimensional micro-focus X-ray computed tomography (3D-mCT) to assess the effects of chronic binge drinking on trabecular parameters and femoral osteometrics. Additionally, the study evaluated the effects of chronic binge alcohol consumption on tensile strength using a three-point bending test.

MATERIAL AND METHOD

Study animals: The research protocol with animal experimentation was approved by the Animal Ethics Committee, University of the Witwatersrand (AESC 2020/ 11/02/C). The study comprised 12 adolescent male Sprague Dawley rats aged 49 days (7 weeks) and weighing approximately 198-250g. According to Sengupta (2013), the age of the rats in this study was 49 postnatal days, which is equivalent to 14.5 years for humans. All study animals were bred and kept at the University of Witwatersrand Research Animal Facility (WRAF), University of the Witwatersrand. These animals were maintained under controlled conditions that are free of most pathogens, a temperature-controlled environment (26-28°C), and a 12-hour light/dark cycle. Study animals were paired together, with free movement within the cages (Length 430mm x width 220mm x height 200mm). All study animals had unrestricted access to tap water and a standard rodent diet.

Group allocation and treatment: A total of 12 adolescent rats were randomly divided into two treatment groups. A 20 % (vol/vol) alcohol solution at a dose of 3g/kg was administered to study animals, as a single dose. This alcohol dose is known to achieve a peak blood alcohol concentration (BAC) of approximately 300mg/dL (Nation *et al*., 1993). Control animals were given an isocaloric equivalent of maltose dextrin. The alcohol/maltose dextrin was administered 3 days/ week by oral gavage (on every alternative day), and no further administration was done during the remaining 4 days of the week. The treatment groups were as follows:

Group A4 (4 weeks alcohol-exposed rats): These rats were administered alcohol for 4 weeks (3 days/ weeks). These are known as chronic binge alcohol-exposed rats.

Group C4 (4 weeks pair-fed control rats): Rats (pair-fed) in this group were given maltose dextrin for 4 weeks, in the same manner as group A4.

Blood samples were drawn from the tail vein an hour after treatment, corresponding with the peak BAC reported by Livy *et al*. (2003), to measure the blood alcohol concentration (BAC). To monitor the study animals' health, parameters including weight and amount of food ingested were recorded.

Skeletal harvesting and Micro-CT. Termination of animals was by pentobarbital intraperitoneal injection on day 28. Immediately after termination, all femora were dissected, and the left bone was then individually wrapped with 0.9 % saline-soaked gauze and stored at -20°C for tensile strength testing. The right bone was placed in 10 % buffered formalin for fixation. Subsequently, a Nikon XTH 225/320 LC X-ray microtomograph was used to obtain 3D-µCT scans of the right femora. The scanning parameters are detailed in Table I.

Table I. Scanning parameters.

Parameter	Value
X-ray voltage	70kV
X-ray current	400µa
Filter	1mm aluminum
Scanning resolution	15µm
Tomographic rotation	360 degrees
Rotation step	1 degree
Frame averaging	4
Scan duration	8 minutes

Femoral osteometry and trabecular morphometry. After reconstruction, data analysis was conducted using VG studio Max® 3.2 software. The femoral osteometry (bone length and bicondylar breadth) was measured using a built-in caliper. The trabecular morphometric parameters of the proximal and distal femur epiphyses were evaluated using the VG studio Max® 3.2 program. They ascertained the trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular spaces (Tb.Sp), and bone volume fraction (BV/TV).

Three-point bending test. To determine tensile strength, a Shimadzu (EZ Test) universal testing machine was used to perform a three-point bending test on the left femora. These bones had been wrapped in gauze soaked in 0.9 % saline solution and kept at -20°C. These bones were defrosted before testing. The stress was applied at the midshaft, or more specifically, at the midpoint between two supports which were 15mm apart. The femora were positioned in such a way as to guarantee bending happened along the anteroposterior plane. Load-displacement curves were recorded until failure at a steady 3mm/min pace.

Data Analysis. The Statistical Package for Social Services (SPSS) version 23 (IBM) program was used to evaluate the data after it was collected and organized in Microsoft Excel 2016 (Microsoft Corporation). The reliability of the data was evaluated using Lin's concordance correlations. The Shapiro-

Wilk test was utilized to assess the data's normality. Because the data were parametric, Levene's test for equality of variances and the Independent Samples t-test for comparing study group means were used. The threshold for significance was set at $p < 0.05$.

RESULTS

Blood alcohol concentration (BAC). The average blood alcohol concentration (BAC) was 106.04 mg/dL (± 17.61) in alcohol groups.

Full bone length and bicondylar breadth. The bone lengths were similar between the alcohol group (mean = 35.18mm ± 0.79) and the pair-fed control group (mean = 35.29mm ± 0.48) ($p = 0.790$). Again, similar group comparison was observed in bicondylar breadth, as both the alcohol group ((mean = 7.33mm ± 0.22) and the pair-fed control groups (mean = 7.37mm ± 0.15) had similar measurements ($p = 0.721$) (Fig. 1).

Trabecular morphometry

Bone to total volume ratio (BV/TV): In the proximal region, the alcohol group exhibited a significantly lower BV/TV (mean = 60.65 % ± 1.31) than the pair-fed control group (mean = 66.73 % ± 2.36) ($p < 0.001$) (Fig. 1a). Conversely, in the distal epiphysis, there was no significant difference in BV/TV between the alcohol group (mean = 27.80 % ± 0.04) and the pair-fed control group (mean = 28.40 % ± 0.04) ($p = 0.800$).

Trabecular Thickness (Tb.Th): In the proximal epiphysis, the alcohol group exhibited thinner trabeculae (mean = 0.109mm ± 0.005) than the pair-fed control group (mean = 0.131mm ± 0.013). This difference was significant ($p = 0.005$) (Fig. 1b). Conversely, in the distal epiphysis, there was no significant difference in trabecular thickness between the alcohol group (mean = 0.079mm ± 0.005) and the pair-fed control group (mean = 0.077mm ± 0.005) ($p = 0.561$) (Fig .1b).

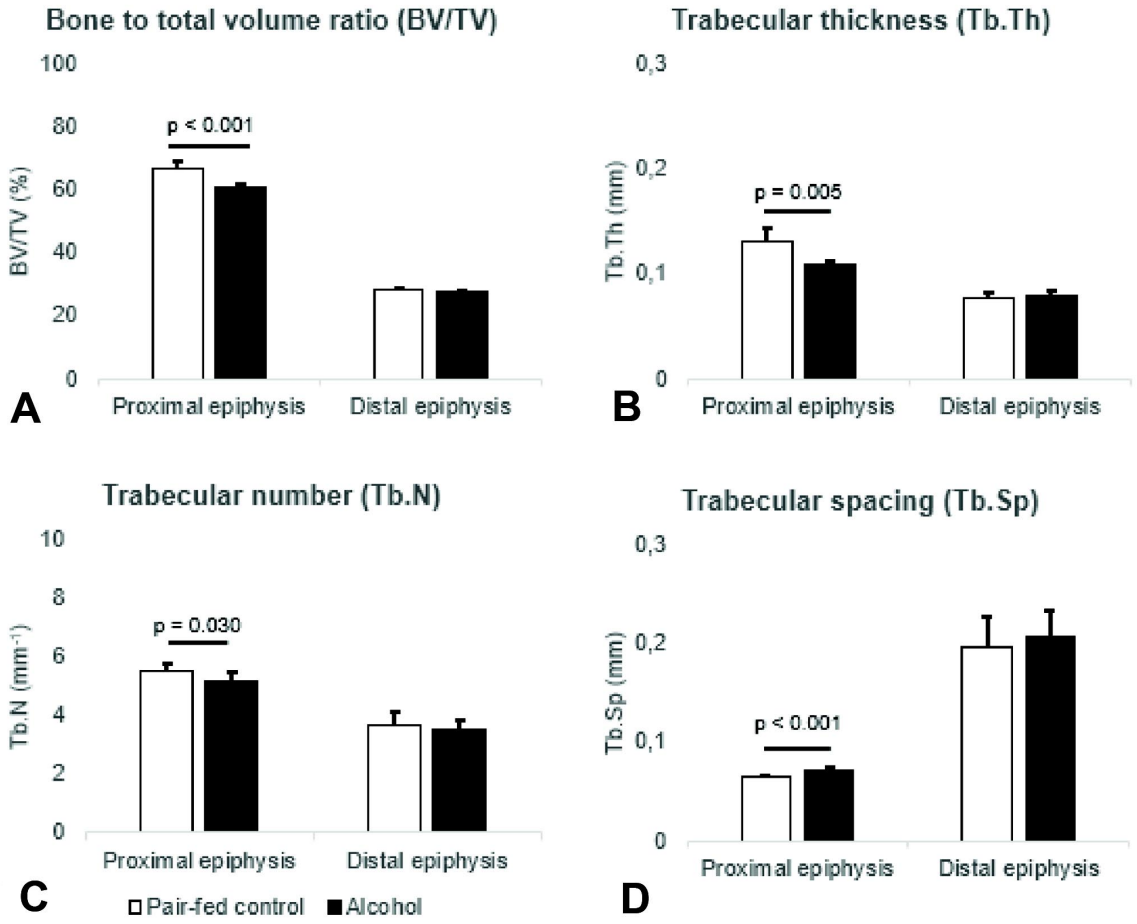

Fig. 1. Trabecular morphometric parameters. Means are given for the pair-fed and alcohol groups; (A), bone to total volumne ratio (BV/TV); (B) trabecular thickness (Tb.Th); (C), Trabecular number (Tb. N) and (D), Trabecular Spacing (Tb. Sp). Error bars represent standard deviation.

Trabecular Number (Th.N): In the proximal epiphysis, the trabeculae were significantly fewer in the alcohol group (mean = 5.138 mm-1 ± 0.326) than the pair-fed control (mean = 5.551 mm-1 ± 0.216) (p =0.030) (Fig. 1c). Conversely, in the distal region, a similar number of trabeculae between the alcohol (mean = 3.529 mm-1 ± 0.315) and the pair-fed control groups (mean = 3.684 mm-1 ± 0.437) was observed (p = 0.49) (Fig. 1c).

Trabecular Spacing (Tb.Sp): In the proximal epiphysis, the trabeculae were significantly wider in the alcohol group (mean = 0.071 mm ± 0.003) than the pair-fed controls (mean = 0.065 mm ± 0.001) (p < 0.001) (Fig. 2). A similar group comparison was seen in the distal epiphysis, with the alcohol group (mean = 0.207 mm ± 0.027) exhibiting wider trabeculae compared to the pair-fed control group (mean = 0.197 mm ± 0.030). However, this difference was not significant (p = 0.590).

Tensile strength: In the chronic binge alcohol consumption model, there was no significant difference in bone weight observed between the alcohol and pair-fed control groups (p = 0.230) (Table II). Furthermore, when examining bone tensile strength parameters, the maximum force and maximum displacement showed similarities between the alcohol and pair-fed control groups (p = 0.94 and 0.72, respectively). Similarly, the maximum time and break force were comparable between the alcohol and pair-fed control groups (p = 0.72 and 0.97 respectively) (Table II).

Table II. Weight measurements and tensile strength parameters of the rat femur.

Parameter		N	Mean	SD
Bone weight (g)	Pair-fed	6	0.995	0.062
	Alcohol	6	0.997	0.103
Maximum force (N)	Pair-fed	6	121.38	11.94
	Alcohol	6	120.89	7.954
Maximum displacement (mm)	Pair-fed control	6	1.082	0.158
	Alcohol	6	1.050	0.139
Maximum time (sec)	Pair-fed	6	21.627	3.168
	Alcohol	6	20.988	2.789
Break force	Pair-fed	6	120.14	13.06
	Alcohol	6	119.14	7.433

DISCUSSION

This study aimed to investigate the effects of chronic binge alcohol consumption on the micro-architecture and tensile strength of the femur in adolescent Sprague Dawley rats. Microfocus X-ray computed tomography was utilized to assess trabecular morphometry in the proximal and distal epiphysis of the femur. Additionally, three-point bending tests were conducted using a universal tensile testing machine to determine the tensile strength of the femur. The findings observed in this study indicated disturbances in the trabecular morphometry of the proximal epiphysis in response to chronic binge alcohol consumption. However, the trabecular morphometry in the distal epiphysis remained unaffected. Interestingly, bone osteometrics and tensile strength were not significantly impacted by chronic binge alcohol consumption.

Based on our findings, the chronic binge alcohol consumption model did not lead to any significant differences in the length and bicondylar breadth of the femur. While we were unable to find comparable studies on the bicondylar breadth of the femur, research investigating the effect of alcohol on the length of long bones (Sampson *et al*., 1996; Simpson *et al*., 2005, Snow & Keiver, 2007) reported a reduction in length in the alcohol-exposed groups. The difference in findings could be attributed to the dosage of alcohol administered to the rats. In the current study, we used 20 % alcohol at 3g/kg body weight, whereas other studies used higher concentrations ranging from 30 % to 36 % in their studies (Sampson *et al*., 1996; Simpson *et al*., 2005; Snow & Keiver, 2007). Furthermore, there appears to be a link between the severity of alcohol's impact on bones and the duration of exposure, with longer periods of alcohol intake resulting in more detrimental effects on bone health (Turner *et al*., 2001). Taken together, the current study suggests that chronic binge alcohol consumption, as administered in our study, may not possess sufficient potency to elicit changes in the osteometry of the adolescent femur seen in previous research (Simpson *et al*., 2005; Snow & Keiver, 2007).

The current study found that chronic binge alcohol drinking perturbs the trabecular morphometry, as alterations in the proximal epiphysis were displayed. We found a decrease in bone tissue volume (BV/TV), thinner trabeculae (Tb.Th), and fewer trabeculae number (Tb.N), which were widely spaced (Tb.Sp). Our findings are consistent with previous research on both young actively growing and adult rats (Sampson *et al*., 1996, 1997; Turner *et al*., 2001; Lauing *et al*., 2008; Maddalozzo *et al*., 2009), which demonstrated alterations such as reduction in bone volume area and the thinning of the trabeculae, following alcohol consumption. These authors reported effects of alcohol on bone trabeculae particularly in the proximal epiphysis of the tibiae (Sampson *et al*., 1996, 1997; Turner *et al*., 2001; Maddalozzo *et al*., 2009). Our study found alterations in the proximal epiphysis with no detrimental influences in the distal epiphysis of the femur in male rats, indicating that alcohol is likely to have a site-specific effect on bones.

Alcohol did not affect bone weight in male rats in our study; this is inconsistent with previous reports which reported a decrease in bone weight. This finding may be attributed to the amount of alcohol that was administered in our study in comparison to other reports (Lauing *et al*., 2008). While no comparable studies addressing maximum displacement and maximum time were found, in the current study maximum force and break force remained unaffected. This finding disagrees with a previous investigation conducted by Hogan *et al*. (1997), for in their study, alcohol consumption led to reduced maximum force and break force across various periods (2, 4, 6, and 8 weeks) (Hogan *et al*., 1997). This discrepancy in findings may be attributed to the varying alcohol dosages administered. In the study of Hogan *et al*. (1997) they employed a modified Lieber-DeCarli diet ad libitum, which contained 35 % alcohol-derived calories. Conversely, our study utilized 20 % vol/vol alcohol administered through oral gavage. This discrepancy in dosage and duration of alcohol may account for the different findings in studies.

CONCLUSION

The current study suggests that prolonged periods of binge alcohol drinking throughout adolescence impair the trabeculae morphometry, which impacts the bone volume fraction and results in fewer and thinner trabeculae in the proximal epiphysis of the femur. Further investigation is warranted to determine the precise mechanisms underlying the adverse effects seen at this level. The results of this study also demonstrate that adolescent binge chronic alcohol consumption may increase the risk of osteoporosis and fractures in later life.

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MNGOMA, N.; PILLAY, D. & BHIKA, A. Efectos del consumo excesivo crónico de alcohol en el desarrollo del fémur de ratas adolescentes Sprague Dawley. *Int. J. Morphol., 42(6)*:1694-1699, 2024.

RESUMEN: Se ha investigado el impacto de la exposición leve, moderada y excesiva al alcohol en la salud ósea. Sin embargo, no existe suficiente literatura sobre los efectos del consumo excesivo crónico de alcohol en la salud ósea de los adolescentes. Por lo tanto, nuestro estudio tuvo como objetivo investigar cómo el consumo excesivo crónico de alcohol afecta el desarrollo de los fémures en ratas adolescentes Sprague Dawley. El estudio se realizó con ratas Sprague Dawley de siete semanas de edad (n = 12), divididas aleatoriamente en dos grupos: el grupo de alcohol, que recibió alcohol al 20 % a 3 g/kg de peso corporal, y el grupo control alimentado en pareja, que recibió un equivalente isocalórico de maltosa dextrina a través de una sonda oral durante cuatro semanas (administrada tres días a la semana en días alternos). Para evaluar las trabéculas tanto en la epífisis proximal como distal y medir las dimensiones corticales del fémur, empleamos la tomografía computarizada de rayos X tridimensional micro-Focus (3D-mCT) y el software volume graphics studio®. Además, se realizó una prueba de flexión de tres puntos para examinar los efectos del alcohol en la resistencia ósea. Los resultados indicaron alteraciones en la morfometría trabecular de la epífisis proximal en ratas expuestas al alcohol. Sin embargo, la morfometría trabecular en la epífisis distal no se vio afectada. Las mediciones osteométricas no mostraron cambios significativos debido al consumo excesivo crónico de alcohol, y la resistencia a la tracción de los huesos tampoco se vio afectada. Estos hallazgos sugieren que el consumo excesivo crónico de alcohol puede tener efectos perjudiciales específicamente en las trabéculas de la epífisis proximal.

PALABRAS CLAVE: Fémur adolescente; Consumo excesivo crónico de alcohol; Epífisis; Resistencia a la tracción; Trabecular.

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Corresponding author:
Dr Akaashni Bhika (BChD, MSc Anatomy)
School of Anatomical Sciences
Faculty of Health Sciences
University of the Witwatersrand
7 York Road
Parktown
Johannesburg 2193
SOUTH AFRICA

E-mail: akaashni.bhika@wits.ac.za