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The Effects of Acute Binge Alcohol Consumption on the Trabecular Morphometry and Tensile Strength of Adolescent Sprague Dawley Rat Femora

Efectos del Consumo Excesivo de Alcohol en la Morfometría Trabecular y la Resistencia a la Tracción en Fémures de Ratas Adolescentes Sprague Dawley

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SUMMARY: Excessive alcohol consumption adversely affects bone metabolism, thus resulting in reduced bone length, density, and strength. Moreover, these deficits in bone density and strength are likely to increase the risk of fragility fractures and the early onset of osteoporosis. While excessive alcohol consumption is an established risk factor for osteoporotic fractures, there remains a dearth of information in literature about bone effects of binge alcohol consumption in adolescents. Therefore, our study aimed to examine the effects of acute binge alcohol consumption on the adolescent bone micro-architecture and tensile strength. Twelve male Sprague Dawley rats aged 7 weeks were randomly placed in 2 groups: alcohol (n =6), receiving alcohol (3g/kg) and pair-fed control (n = 6), receiving an isocaloric equivalent of maltose dextrin via oral gavage for 3 days in one week (on alternative days). The femora were dissected and scanned using a Micro-Focus X-ray Computed Tomography (3D-µCT). Following reconstruction, trabecular morphometry was assessed in both the proximal and distal epiphysis, using a Volume Graphics Studio® software. A three-point bending test was employed to examine the effect of alcohol on the tensile strength of the bone. Results showed trabeculae parameters to be affected in the distal epiphysis of the femur, while in the proximal epiphysis it remained unaffected. Tensile strength parameters were also not affected by the consumption of alcohol. These findings may suggest that acute binge alcohol consumption has detrimental effects on the bone micro-architecture specific to the distal epiphysis.

KEY WORDS: Adolescent; Acute binge; Alcohol; Trabeculae; Femur.

INTRODUCTION

Drinking alcohol is a common practice globally in various social and cultural settings. There are various alcohol drinking patterns such as occasional, heavy chronic, and binge drinking. Binge drinking is defined as consuming a large amount of alcohol in a short period and is known to be a common practice amongst adolescents (Mmereki *et al*., 2022; Morojele & Ramsoomar, 2016). Adolescence is a crucial time of attaining peak bone mass, which serves as a buffer against osteoporosis and fractures in adulthood (Weaver *et al*., 2016).Impaired bone development during this stage can have long term implications on osseous tissue health and increase the risk of osteoporosis and fractures later in life (Abrams, 2003).

To date, the effects of acute binge drinking on adolescent long bones have been investigated in a limited number of studies. Much of the literature on the effects of alcohol on bone development have focused on chronic alcohol consumption rather than acute binge drinking episodes. These studies have demonstrated that alcohol inhibits bone growth, decreases bone mineral density, and adversely affects the trabecular bone architecture (Sampson *et al*., 1996; Hogan *et al*., 1997; Sampson *et al*., 1997; Ferreira *et al*., 2022; Pillay & Ndou, 2022). Hogan *et al*. (1997), reported reduced biomechanical strength in bone following chronic alcohol exposure. Additionally, Lauing *et al*. (2008), reported a decrease in tibial cancellous bone mineral density after acute alcohol exposure. However, little research has been done to explore the effects of acute binge alcohol consumption on the development of adolescent bone with reference to the femur which is the longest bone in the body forming important articulations both proximally and

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distally. A better understanding of the effects of acute binge drinking on the adolescent long bone such as the femur can help identify potential intervention strategies and promote awareness about risks associated with excessive alcohol consumption on growth and development.

Binge drinking amongst adolescents is a public concern due to its potential negative effects on physical and mental health. Understanding the impact on bone health adds to the evidence of addressing the issue and can contribute to policy development and interventions aimed at reducing alcohol consumption in this vulnerable population.

Thus, the aim of the study was to evaluate the effect of acute binge alcohol consumption in growing rats on longitudinal growth of the femur and parameters of bone strength. This was achieved by using three-dimensional micro-focus X-ray computed tomography (3D- mCT) to assess the bone to total volume ratio, trabecular thickness, spacing and trabecular number in both the proximal and distal epiphysis of the femur as well as femur osteometrics. The effects of acute binge alcohol consumption on tensile strength was assessed using a 3-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). Every effort was made to minimise suffering. A total of 12 adolescent male Sprague Dawley rats aged 49 days (7 weeks) and weighing between 261-320g were used in this study. The age of rats in this study (49 postnatal days) was equivalent to 14.5 human years (Sengupta, 2013). All study animals were bred and kept at the University of the Witwatersrand Research Animal Facility (WRAF).These animals were maintained under controlled conditions that are free of most pathogens, in 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 430 mm x width 220 mm x height 200 mm). All study animals had unrestricted access to tap water and a standard rodent diet.

Group allocation and treatment: Study animals were randomly allocated to 1 of the 2 treatment groups, with 6 rats per group. A 20 % (vol/vol) alcohol solution at a dose of 3 g/kg was administered to study animals, as a single dose via oral gavage. This alcohol dose is known to achieve a peak blood alcohol concentration (BAC) of approximately 300 mg/dL (Nation *et al*., 1993). Control animals were given an isocaloric equivalent of maltose dextrin via oral gavage. The alcohol/maltose dextrin was administered 3 days/week (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 A1 (1-week alcohol-exposed rats): Rats in this group were administered alcohol for 3 days of the week (every alternate day). These are known as the acute binge alcohol-exposed rats.

Group C1 (1-week pair-fed control rats): As a caloric equivalent control (pair-fed), these rats were given maltose dextrin instead of alcohol for 1 week (3 days of the week), on the same days as Group A1.

To monitor blood alcohol concentration (BAC), tail vein blood was sampled, an hour after treatment, when peak BAC is achieved (Livy *et al*., 2003). Parameters such as weight and food consumed were recorded to track the health of the study animals.

Skeletal harvesting and Micro-CT: Study animals were terminated by pentobarbital intraperitoneal injection on day 7. Immediately after termination, all femora were dissected, the left bone was then individually wrapped with 0.9 % saline-soaked gauze and stored at -20 °C for biomechanical analysis. The right bone was placed in 10 % buffered formalin for fixation. Subsequently, a Nikon XTH 225/320 LC X-ray Microtomograph was used for obtained 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	1 mm aluminium
Scanning resolution	15µm
Tomographic rotation	360 degrees
Rotation step	1 degree
Frame averaging	4
Scan duration	8 minutes

Femoral osteometry and trabecular morphometry: Following reconstruction, the VG studio Max® 3.2 software was employed for data analysis. A built-in calliper was used to determine femoral osteometrics (bone length and bicondylar breadth). The VG studio Max® 3.2 software was used to assess trabecular morphometric parameters in both the proximal and distal epiphysis of the femur. These determined bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th) and trabecular spaces (Tb.Sp).

Three-point bending test: As previously mentioned, the left bones were individually wrapped with gauze soaked in 0.9 % saline solution and stored at a temperature of -20°C. On the day of the three-point bending test, these bones were thawed. The three-point bending test was performed on the left femora of each rat. The load was applied at the midshaft, which is the point halfway between two supports measuring 15 mm apart. The femora were positioned to ensure that bending occurred along the anteroposterior plane. Load-displacement curves were recorded with a constant speed of 3 mm/min until failure occurred.

Data analysis: Data obtained was managed in Microsoft Excel 2016 (Microsoft Corporation) and analysed using the Statistical Package for Social Services (SPSS) version 23 (IBM) software. To assess the reliability of the data, Lin's concordance correlations for reliability were employed. Normality of the data was examined using the Shapiro-Wilk's test. Given the parametric nature of the data, the Levene's test for equality of variances was employed alongside the Independent Samples t-test for comparing means between the study groups. The significance level was set at $p < 0.05$.

RESULTS

Measurement reliability: Measurement reliability was assessed using Lin's concordance correlation coefficient (pc), which yielded values above 0.7 for all measurements. These results indicate a high degree of measurement reliability, suggesting that the measurements are consistent and dependable.

Blood alcohol concentration: The average blood alcohol concentration was 108.04 mg/dL (± 16.60) in alcohol groups.

Full bone length and bicondylar breadth: Similar bone length was displayed between the alcohol (mean = 33.25 mm ± 0.77) and the pair-fed control groups (mean = 33.11 mm ± 0.59) (p = 0.66) (Fig. 1). This pattern continued in bicondylar breadth values, as both the alcohol group (mean = 7.45 mm ± 0.15) and the pair-fed control (mean = 7.32 mm ± 0.22) displayed similar measurements (p = 0.24) (Fig. 1).

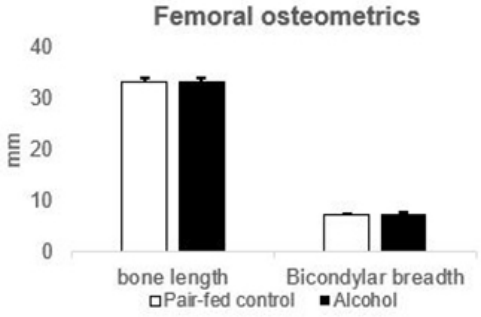

Fig. 1. Femoral osteometrics. Mean values for bone length and bicondylar breadth are given for the alcohol and pair-fed control. Error bars represent standard deviation.

Trabecular morphometry

Bone to total volume ratio (BV/TV): No significant group difference in bone to total volume ratio (BV/TV) was observed in the proximal epiphysis, however in the distal epiphysis the alcohol group had a lower BV/TV. The alcohol group (mean = 52.44 % ± 1.21) appeared to have a lesser BV/TV than the pair-fed control group (mean = 55.29 % ± 3.44) in the proximal epiphysis, however no statistical significance was detected (p = 0.084) (Fig. 2a). In the distal region, the BV/TV was significantly lower in the alcohol group (mean = 20.69 % ± 1.77) compared to the pair-fed control (mean = 28.51 % ± 2.40) (p = 0.002) (Fig. 2a).

Trabecular Thickness (Tb.Th): The trabeculae were thinner for the alcohol group in both the proximal and distal epiphysis of the femur. In the proximal limb, the alcohol group (mean = 0.086 mm ± 0.006) exhibited thinner trabeculae than the pair-fed control (mean = 0.098 mm ± 0.010) (p = 0.033) (Fig. 2b). This pattern continued in the distal epiphysis, with the alcohol (mean = 0.066 mm ± 0.002) displaying thinner trabeculae compared to that of the pair-fed control group (mean = 0.080 mm ± 0.002) (p = 0.023) (Fig. 2b).

Trabecular Number (Tb.N): In the proximal epiphysis, the alcohol group (mean = 6.122 mm-1 ± 0.) had a marginally higher trabecular number than the pair-fed control (312mean = 5.669 mm-1 ± 0.479). This difference was significant (p = 0.044) (Fig. 2c). Conversely, in the distal epiphysis, the trabecular number was significantly lower in the alcohol group (mean = 3.128 mm-1 ± 0.248) than the pair-fed control (mean = 3.675 mm-1 ± 0.0298) (p = 0.030) (Fig. 2c).

Trabecular Spacing (Tb.Sp): No group differences in trabecular spacing (Tb.Sp) were observed in both limbs (proximal and distal). In the proximal end, the trabecular spacing was similar between the alcohol (mean = 0.078 mm ± 0.002) and the pair-fed control group (mean = 0.079 mm ± 0.008) (p = 0.070) (Fig. 2d). Conversely, in the distal region, the observation of significantly wider trabeculae was seen in the alcohol group (mean = 0.255 mm ± 0.024) than the pair-fed control group (mean = 0.196 mm ± 0.018) (Fig. 2d) (p = 0.008).

Three-point bend testing of the femur, tensile strength assessment. In the acute binge alcohol consumption model, no significant difference in bone weight was observed between the alcohol and pair-fed control group (Table II) With respect to bone tensile strength parameters, the

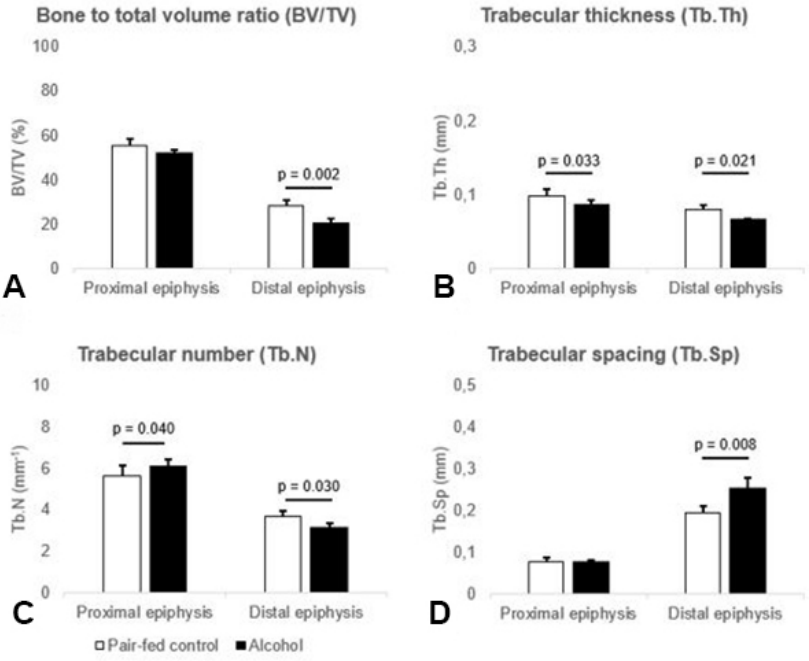

Fig. 2. Trabecular morphometric parameters. Means are given for the pair-fed and alcohol groups; (A), bone to total volume ratio (BV/TV); (B), trabecular thickness (Tb.Th); (C), trabecular number (Tb.N) and (D), Trabecular Spacing (Tb.Sp). Error bars represent standard deviation.

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

Parameter		N	Mean	SD
Bone weight (g)	Pair-fed control	6	0.872	0.088
	Alcohol	6	0.887	0.065
Maximum force (N)	Pair-fed control	6	83.527	14.336
	Alcohol	6	84.692	9.047
Maximum displacement (mm)	Pair-fed control	6	1.200	0.457
	Alcohol	6	1.192	0.363
Maximum time (sec)	Pair-fed control	6	23.993	9.145
	Alcohol	6	23.823	7.257
Break force	Pair-fed control	6	81.663	13.993
	Alcohol	6	78.379	8.543

maximum force, maximum displacement, and maximum time were similar between the alcohol and the pair-fed control groups (p = 0.87, 0.97 and 0.97, respectively) (Table II). The break force was marginally lower in the alcohol group compared to the pair-fed control group; however, this difference was not statistically significant (p = 0.63).

DISCUSSION

The aim of this study was to examine the effects of acute binge alcohol consumption on the micro-architecture and tensile strength of the femur in adolescent Sprague Dawley rats. We employed micro focus X-ray computed tomography to assess trabecular morphometry in the proximal and distal epiphysis of the femur and osteometric parameters. Tensile strength parameters were determined by the use of a 3-point bending test. This was to determine whether an acute binge alcohol drinking model could result in weaker bones. The study found trabeculae parameters to be negatively affected in the distal epiphysis, while both the osteometric and tensile strength parameters remained unaffected.

In the current study, the acute binge alcohol consumption model exhibited no significant changes in the length and bicondylar breadth of the femur between study groups. This finding is similar to a study by Sampson *et al*. (1996) which showed the femora treated with alcohol exhibiting similar lengths to the pair-fed controls after two weeks of alcohol administration. However, in the same study when alcohol was given for a longer period this resulted in shorter femora (Sampson *et al*., 1996). This suggests that if alcohol is given for a longer period this may result in a negative impact on bone length (Turner *et al*., 1987, 2001). Considering the duration of the acute alcohol consumption in the current study, it is plausible to propose that acute binge alcohol consumption duration was too short to cause any detrimental effects on the osteometry of the adolescent femur. This finding also corroborates with a study by Turner *et al*. (1988), which found no changes in femoral length in alcohol-treated rats compared to pair-fed controls, following acute administration of alcohol (Turner *et al*., 1988).

Regarding, trabecular morphometry it was found that acute binge alcohol consumption caused detrimental effects on the internal architecture and structure of the trabecular bone in the distal epiphysis. These alterations were characterized by a decrease in bone tissue volume (BV/TV), thinner trabeculae (TbSp), fewer trabeculae (TbN) and a decrease in spacing. However, this study found no negative influence of acute binge alcohol consumption in the proximal end of the femur, except for a reduction in trabecular thickness, which was observed in the alcohol group. Our findings concur with previous studies on actively young growing rats (Sampson *et al*., 1996, 1997) and in adult rats (Turner *et al*., 2001; Maddalozzo *et al*., 2009)

which reported changes such as a decrease in bone fraction area (BV/TV), trabecular thinning (TbTh), however, these authors reported the effects of alcohol exposure in the proximal limb of the osseous tissue (tibia). The higher proportion of trabecular bone and increased mechanical loading in the distal end of the femur maybe likely contributing factors to why alcoholaffected trabecular bone in the distal region of male rats in our study more than the proximal end (Kivell, 2016).

No significant differences in the bone weight and tensile strength properties were observed in the current study. The maximum displacement and maximum time results from the present study could not be compared directly to the other studies as no previous studies can be found in the literature that include the effects of acute binge alcohol consumption on these properties. However, Hogan *et al*. (1997), using a chronic model did investigate the effects of alcohol consumption on both the maximum force and break force and reported a lower maximum force in alcohol-exposed animals compared to the pair-fed controls at all time periods. This trend was also observed in the break force results (Hogan *et al*., 1997). This discrepancy in findings is likely attributed to differences in study design. In contrast to Hogan *et al* (1997), who used three-week-old female Sprague-Dawley rats and fed them a modified Lieber-DeCarli diet ad libitum containing 35 % alcohol-derived calories for eight weeks, we administered 20 % alcohol via oral gavage to seven-week-old male and female Sprague-Dawley rats for one week in our study. It is possible that if we had used a higher alcohol concentration, and a longer administration period, our results would have been similar to those of Hogan *et al* (1997).

CONCLUSION

Our findings provide new evidence that acute binge alcohol consumption, during adolescence, impairs the trabeculae morphometry affecting the bone volume fraction, causing fewer and thinner trabeculae in the distal epiphysis of the femur.

The exact mechanisms involved in the deleterious effects observed at this dose should be investigated in further research, but our results highlight the intake of alcohol in early adolescence may be a risk for osteoporosis and fractures later in life.

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RESUMEN: El consumo excesivo de alcohol afecta negativamente al metabolismo óseo, lo que resulta en una reducción de la longitud, densidad y resistencia de los huesos. Además, es probable que estos déficits en la densidad y la fuerza ósea aumenten el riesgo de fracturas por fragilidad y la aparición temprana de osteoporosis. Si bien el consumo excesivo de alcohol es un factor de riesgo establecido para las fracturas osteoporóticas, existe escasa información en la literatura sobre los efectos óseos del consumo excesivo de alcohol en adolescentes. Por lo tanto, nuestro estudio tuvo como objetivo examinar los efectos del consumo excesivo de alcohol en la microarquitectura ósea y la resistencia a la tracción e n ratas adolescentes. Doce ratas macho Sprague Dawley de 7 semanas de edad se colocaron aleatoriamente en 2 grupos: alcohol (n = 6), que recibieron alcohol (3 g/kg) y control (n = 6), que recibieron un equivalente isocalórico de maltosa dextrina mediante sonda oral, durante 3 días en una semana (en días alternos). Los fémures se diseccionaron y escanearon mediante una tomografía computarizada de rayos X con microenfoque (3D-mCT). Después de la reconstrucción, se evaluó la morfometría trabecular tanto en la epífisis proximal como en la distal, utilizando un software Volume Graphics Studio®. Se empleó una prueba de flexión de tres puntos para examinar el efecto del alcohol sobre la resistencia a la tracción del hueso. Los resultados mostraron que los parámetros de las trabéculas se vieron afectados en la epífisis distal del fémur, mientras que en la epífisis proximal no se observaron afectados. Los parámetros de resistencia a la tracción tampoco se vieron afectados por el consumo de alcohol. Estos hallazgos pueden sugerir que el consumo excesivo de alcohol tiene efectos perjudiciales sobre la microarquitectura ósea específica de la epífisis distal del hueso.

PALABRAS CLAVE: Ratas adolescente; atracones agudos; Alcohol; trabéculas; Fémur.

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