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Examining the Impact of Acute Binge Alcohol Consumption on Trabecular Morphometry and Tensile Strength in Adolescent Sprague Dawley Rat Mandibles

Análisis del Impacto del Consumo Excesivo de Alcohol en la Morfometría Trabecular y la Resistencia a la Tracción en Mandíbulas de Ratas Adolescentes Sprague Dawley

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SUMMARY: Underage drinking has become a major public concern having a negative impact on the growth and development of the skeleton. Peak bone mass is attained during adolescence hence the aim of the study was to investigate the effect of acute binge alcohol consumption on trabecular morphometry and tensile strength of the adolescent mandible in the Sprague Dawley (SD) rat. The study comprised of 24 SD rats, aged 7 weeks, placed into either the alcohol-exposed [n=12 (6 males and 6 female)] or pair-fed control group [n=12 (6 male and 6 female)]. The treatment of the groups was as follows; the alcohol exposed group and the pair-fed control were administered a single daily dose of 3 g/kg of 20 % alcohol 3 days a week (alternate days) for 7 days and a caloric equivalent dose of maltose dextrin via oral gavage, respectively. The animals were terminated on day 7 via pentobarbital injection. The mandibles were harvested and scanned using a Nikon XTH 255L 3D-microCT scanner (Nikon Metrology, Leuven, Belgium), and biomechanical tests were done using a Shimadzu universal tensile strength testing machine (China). Following scanning and reconstruction, the trabecular morphometry was assessed using Volume Graphics Studio® software. A 3-point bending test was used to evaluate the tensile strength of the bone. Findings from our study showed changes in some trabecular parameters in the female alcohol-exposed group, while the male groups remained unaffected. No changes in tensile strength were seen when comparing male pair-fed control and alcohol-exposed groups and when comparing female pair-fed control and alcohol-exposed groups. Trabecular and tensile strength differences were observed between the sexes when comparing male pair-fed control and alcohol-exposed groups to female pair-fed control and alcohol-exposed groups. These findings do suggest that acute binge alcohol consumption has detrimental effects on the bone micro-architecture in female alcohol-exposed rats and that differences are seen between the sexes.

KEY WORDS: Adolescent; Acute binge; Alcohol; Biomechanics; Mandible.

INTRODUCTION

Binge drinking of alcohol is becoming a common practice amongst adolescents in South Africa and a major public concern. Binge drinking or heavy episodic drinking is defined as the consumption of ≥ 5 drinks for males and ≥ 4 drinks for females on one occasion or during one session of drinking, or the consumption of ≥ 60 g of pure alcohol at least once per month (Morojele & Ramsoomar, 2016).

It has been established that chronic alcohol exposure in adults increases the propensity of osteoporosis resulting in a decrease in bone density and an increased risk of fractures. However, there is a gap in our knowledge regarding the effects of binge alcohol consumption on the adolescent skeleton (Sampson *et al*., 1996; Sampson, 1997). Even less, is known on the effect of binge alcohol consumption on the adolescent mandible.

There have been animal studies on the effects of alcohol consumption on long bones in adolescence. The findings observed in these studies include a significant decrease in bone growth, volume, density and strength (Sampson, 1997; Hogan *et al*., 1999; LaBrie *et al*., 2018). Also, Foger-Samwald *et al*. (2018), reported on the effects of binge alcohol consumption using prepubescent pigs as a

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model. The results showed a decrease in serum calcium and phosphate levels and the femur showed decreased density and fewer trabeculae (Föger-Samwald *et al.,* 2018). The impact of binge drinking on trabecular parameters and on the tensile strength of adolescent bone is still limited, especially in the craniofacial bones, warranting further investigation.

The mandible is an unpaired bone of the craniofacial skeleton. This bone is essential for mastication and is also involved in processes such as speech, facial expression, and dentition (Breeland *et al.*, 2022). The growth and development of the mandible requires a control of cell proliferation, differentiation, and the ossification processes. Alcohol consumption is known to disturb these processes (Durham *et al*., 2019). Therefore, this study investigated the effects of acute binge alcohol consumption on the internal architecture of adolescent Sprague Dawley rat mandibles. The study employed the use of microtomography to study the effects of alcohol on trabecular morphology and 3-point bending tests for tensile strength of this osseous tissue.

MATERIAL AND METHOD

Study animals. The study received ethical approval from the Animal Research Ethics Committee, University of the Witwatersrand (AREC 2020/11/02C). 24 SD rats (male and female) aged 7 weeks, were placed into either the alcohol-exposed [n=12 (6 males and 6 female)] or pair-fed control group [n = 12 (6 male and 6 female) at 7 weeks of age weighing approximately 175 g-199 g. All study animals were bred and kept at the University of the Witwatersrand Research Animal Facility (WRAF), Parktown Campus. These animals were maintained under controlled conditions that were free of most pathogens, in a temperature-controlled environment (26-28 °C ± 2 °C) and a 12-hour light/dark cycle. The rats were housed in pairs (according to sex) in plastic cages of 43 mm long, 220 mm wide and 200 mm height with free movement within the cages. All the animals in the study were fed a standard rodent diet and water was provided *ad libitum.*

Group Allocation

Alcohol-exposed rats (6 male and 6 female rats). These animals were exposed to alcohol for 1 week mimicking an acute binge alcohol model. Alcohol was administered by a single daily dose via oral gavage of a 20 % (vol/vol) alcohol solution at a dose of 3 g/kg. Blood alcohol concentrations (BACs) were tested an hour after exposure by drawing blood using the tail prick method. Blood was stored in heparinized microcapillary tubes (Marienfeld). BACs were tested using an alcohol colorimetric assay kit (Sigma-Aldrich) and readings were done using an absorbance reader at a wavelength of 630 nm. Alcohol was given 3 days/week (on every alternate day). No alcohol was administered during the remaining 4 days of the week.

Pair-fed control rats (6 male and 6 female rats). As a caloric equivalent control, there was a pair-fed rat that was matched individually to an alcohol-fed rat based on initial body weight. The pair-fed group was given an isocaloric equivalent of maltose dextrin (Sigma-Aldrich) which was also administered via oral gavage.

Termination and skeletal harvesting. The animals were terminated on day 7 by pentobarbital intraperitoneal injection. The mandibles were then harvested and stored in 10 % buffered formalin for further fixation and processing.

Three-dimensional micro-computed tomography scanning. A Nikon XTH 225/320 LC X-ray microtomograph was used for computed tomography (3D-mCT) using the following parameters: X-ray voltage of 100 kv, X-ray current of 100 ma, a scanning resolution of 15 mm, and a scan duration of 20 minutes. VG studio Max® 3.5 software was used for analysis of the following parameters: bone volume to total volume ratio (BV/TV), trabecular thickness (Tb/Th), trabecular number (TbN), and trabecular spaces (TbSp). The region of interest (ROI) selected was between the 1st and 2nd molar teeth in the body of the mandible.

Biomechanical testing. 3-point bending tests using a Shimadzu universal testing machine were done. The specimens (left hemi-mandibles) were placed on two rounded bars set 15 mm apart and a load was applied. The following parameters were tested: bending strength which is the amount of force required for the bone to start to bend; deflection which is the amount of vertical deviation from the horizontal plane required to facture the mandible; ultimate load which is the amount of force required to fracture the mandible; and time which refers to the duration that the mandible withstands the force until fracture.

Data analysis. Data obtained was managed in Microsoft Excel 2021 (Microsoft Corporation). The data was analyzed using the Statistical Package for Social Service (SPSS) version 28 (IBM ®), 2021 software. The reliability of the data was observed by using Lin's concordance correlations for reliability. Normality of the data was tested using the Shapiro-Wilk test. The t-test was completed on normally distributed data. Where data was non-parametric the Mann-Whitney U test was completed. A p-value ≤ 0.05 was considered statistically significant, at a confidence interval of 95 %.

RESULTS

Blood Alcohol Concentration. The mean blood alcohol concentration (BAC) was 108.04. mg/dl (± 16.60) in the alcohol exposed rats and negligible in the pair-fed control rats.

Trabecular Morphometry

Bone to Total Volume Ratio (BV/TV). Males and females exhibited differences in the bone to total volume ratio (BV/TV). The BV/TV in males was marginally lower in the pair-fed control group (mean = 58.40 % ± 3.65) than the alcohol-exposed group (mean = 60.88 % ± 6.53) (p = 0.223). Conversely, in the female rats, the pair-fed control group (mean = 68.27 % ± 4.85) had a significantly higher BV/TV than the alcohol-exposed group (mean = 61.20 % ± 3.45) (p = 0.011). The BV/TV between the male and female pair-fed controls was statistically different, with the female controls BV/TV being greater than the BV/TV in males (p = 0.02). The BV/TV between male and female alcohol-exposed groups showed no statistical differences (p = 0.458) (Fig. 1A).

Trabecular Thickness (TbTh). With regards to trabecular thickness similar patterns were observed in males and females. The pair-fed control groups had thicker trabeculae in both sexes. Trabeculae were thinner in the male alcohol-exposed group (mean = 0.14 mm ± 0.03) than the pair-fed control group (mean = 0.16 mm ± 0.03) however, no significance was detected (p= 0.140). Thicker trabeculae were observed in the female pair-fed control group (mean = 0.18 mm ± 0.03) than the alcohol-exposed group (mean = 0.16 mm ± 0.01), but this difference was not significant (p = 0.180). Similarities were exhibited between the male and female pair-fed and the alcohol groups (p = 0.124 and p = 0.134, respectively) (Fig. 1B).

Trabecular Number (TbN). The trabecular number in the mandible for the both the male and female rats was similar between the study groups. The trabecular number (TbN) in the male pair-fed control and alcohol-exposed groups were similar (mean = 3.63 mm-1 ± 0.38 and mean = 3.86 mm-1 ± 0.42, respectively) (p = 0.186). Again, in the female rats the pair-fed control and the alcohol-exposed groups exhibited similarities (mean = 3.76 mm-1 ± 0.37 and mean = 3.84 mm-1 ± 0.18, respectively) (p = 0.329). Also, trabecular distribution showed no differences between male and female pair-fed control groups (p = 0.302) as well as between male and female alcohol-exposed groups (p = 0.462) (Fig. 1C).

Trabecular Spacing (TbSp). Trabecular spacing stratified by study group showed a varied pattern in the mandible. In the male rats, the trabecular spacing was not statistically different for the pair-fed control compared to the alcohol-exposed rats (mean = 0.12 mm ± 0.01 and mean = 0.12 mm ± 0.05, respectively) (p = 0.240). Conversely, the female rats, exhibited significantly wider trabeculae in the alcohol-exposed group than the pair-fed control group in females (mean = 0.1 mm ± 0.01 and mean = 0.08 mm ± 0.01) (p = 0.014). A significant difference was exhibited in trabecular spacing (TbSp) between the male and female pair-fed control groups (p < 0.001). However, no significant differences in TbSp were observed between male and female alcohol-exposed groups (Fig. 1D).

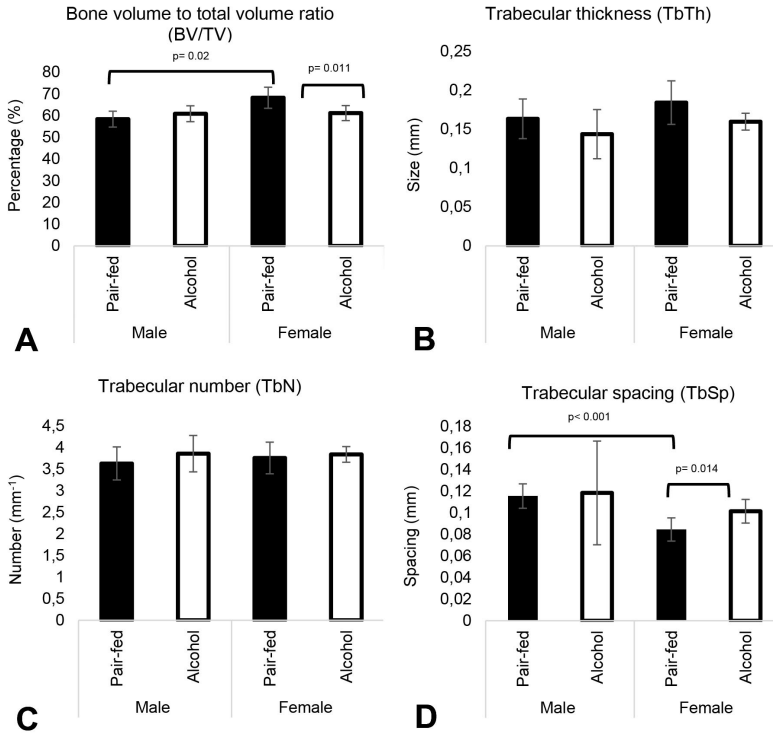


Fig. 1. Trabecular morphometric parameters: Trabecular morphometric parameters for male and female rats, value are represented as means; A. Bone tototal volume(BV/TV); B. Trabecular thickness (TbTh); C. Trabecular number (TbN); D. Trabecular spacing (TbSp). Error bars represent standard deviation.

Three-point bending testing of the mandible, tensile strength assessment. Regarding bone tensile strength parameters, the maximum force, break force, maximum displacement, and maximum time were similar between the alcohol-exposed and the pair-fed control groups in the male (p = 0.102, p = 0.132, p = 0.132, and p = 0.132, respectively) and female groups (p = 0.065, p = 0.057, p = 0.294, and p = 0.294, respectively) (Table I).

Table I. Tensile strength of the mandible in male and female rats in an acute binge alcohol model.

		Males			Females		
		N	Mean	SD	N	Mean	SD
Maximum force (N)	Pair-fed control	6	57.27	2.62	6	49.30	13.49
	Alcohol	6	66.50	16.44	6	37.96	9.99
Maximum displacement (mm)	Pair-fed control	6	6.23	0.22	6	6.11	0.27
	Alcohol	6	5.22	2.14	6	6.00	0.39
Maximum time (sec)	Pair-fed control	6	124.68	4.38	6	122.14	5.38
	Alcohol	6	121.05	5.53	6	119.97	7.81
Break force (N)	Pair-fed control	6	56.01	2.73	6	47.24	13.70
	Alcohol	6	64.98	16.92	6	31.95	16.72

No significant differences were seen in the maximum force (p= 0.093), break force (p = 0.077), maximum displacement (p = 0.195), and maximum time (p = 0.195) between male and female pair-fed control animals. The maximum force (p = 0.002), and the break force (p = 0.003) were significantly higher in the male alcohol-exposed rats than the female alcohol-exposed rats. A comparison between the male and female alcohol-exposed rats, displayed no significant differences in the maximum displacement and maximum time (p = 1.0 for both comparisons, respectively).

DISCUSSION

We sought to understand the effect that binge alcohol exposure would have on the trabecular morphology and the tensile strength of the adolescent rat mandible. Three-dimensional micro-focus X-ray computed tomography (3D-μCT) was used to access trabecular volume, thickness, number, and spacing in the mandible. Altered trabecular parameters were observed with regards to bone volume ratio and trabeculae spacing in female rats. The tensile strength remained unaffected for most parameters except for an increase in the maximum force, and the break force which were significantly higher in the male alcohol-exposed rats than in the female alcohol-exposed rats.

In the present study, the bone to total volume ratio (BV/TV) was lower in the alcohol group in female rats. When comparing the two sexes, the BV/TV was greater in the female pair-fed control rats than in the male pair-fed control rats. A decrease in BV/TV was observed in alcohol-exposed rats in a study conducted by Sampson *et al*. (1998b), where the effect of alcohol consumption on adult and aged bone in a rat model was studied. Likewise, a decrease was observed in BV/TV in the alveolar bone of female adolescent rats after 30 and 60 days of alcohol exposure (Maia *et al*., 2021). Callaci *et al*. (2004), reported that binge drinking had no effect on male rats in the first 2 weeks of exposure but observed a decrease in BV/TV after 3 weeks of alcohol exposure. Our study indicates that binge alcohol-exposure for a period as short as one week affects the BV/TV of female rats but has no significant impact on males. The duration of exposure may be the reason for no noticeable changes in the male rats. The differences seen between male and female rats, where the female pair-fed control group in our study had a greater BV/TV than the male pair-fed control group may be attributed to female rats having high serum oestrogen levels during adolescence which may have a positive impact on bone development (Schiessl *et al*., 1998).

Trabecular thickness (TbTh) showed no differences between the male pair-fed control and alcohol-exposed groups. Similarities were also observed between female pair-fed control and alcohol exposed-groups. No sex differences were observed between male and female pair-fed control and alcohol exposed groups. Our finding corroborates with a study by Sampson *et al*. (1998b) with no differences in TbTh between the pair-fed and alcohol exposed rats. However, Maia *et al*. (2021), observed thinner trabeculae in alveolar bone of adolescent female rats exposed to a binge alcohol model. There were differences in study designs between our study and Maia *et al*. (2021), which could explain the difference in findings. In the study by Maia *et al*. (2021), the animals were exposed to 3 g/kg of a 20 % vol/vol of alcohol for 4 weeks (3 days per week). We used the same dosage and volume of alcohol as Maia *et al*. (2021), however our animals were only exposed to a shorter duration of alcohol exposure [7 days (3 days per week)], which, may suggest why there were no changes in trabecular thickness observed.

No differences in trabecular number (TbN) were observed in our study which corroborates with a study conducted by Maia *et al*. (2021). However, Sampson *et al*. (1998b) and Föger-Samwald *et al*. (2018) observed a decrease in TbN in animals exposed to alcohol. The differences exhibited may be attributed to variances in study design, and the duration of exposure of alcohol to the osseous tissue.

Wider trabecular spaces (TbSp) were observed in the female alcohol-exposed group than the pair-fed control. This finding is similar to the previous reports which also exhibited wider trabecular spacing in alcohol exposed animals

(Sampson *et al*., 1998a; Maia *et al*., 2021). Wider trabecular spaces can be linked to weaker bones (Pillay & Ndou, 2021). Wider trabeculae were exhibited in the male pair-fed controls than the female pair-fed controls. The wider spaces seen in the male pair-fed controls may be due to female rats having high serum oestrogen levels during adolescence, which supports the idea that oestrogen can affect human bone strength and mass by lowering the remodelling threshold, which may have had a positive impact on bone development (Schiess *et al*., 1998). The onset of puberty in males is also later than it is in females, thus these changes could be attributed to females entering puberty before males and thus skeletal development taking place before males.These changes in TbSp may be related to the stage of puberty that the animals are in and may change as puberty progresses. Sex differences in bone mass, width, length, and strength occur later on in puberty (Wang & Seeman, 2008).

Regarding tensile strength parameters, in the present study, no statistical differences in bone strength was observed between the male pair-fed control and alcohol-exposed groups. Also, no differences were exhibited between the female pair-fed control and alcohol-exposed exposed groups. This finding is similar to a study conducted by Callaci *et al*. (2006), which exhibited no significant effects on vertebral compressive bone strength in male Sprague Dawley rats exposed to binge alcohol exposure after 1 and 2 weeks of exposure, whereas animals exposed to alcohol for 3 weeks showed significant effects on vertebral compressive strength. The one-week duration of alcohol exposure in our study and the one and two weeks of Callaci *et al*. (2004), may suggest why the tensile strength of the bone was not affected due to the duration of exposure. This may also indicate that a loss of trabecular and cortical bone occurs after an increased exposure to a binge drinking model (3 weeks or longer). Longer exposure of alcohol may have had a more adverse effect on the bone strength. We could not find comparable studies that investigated tensile strength of the mandible.

Furthermore, our study showed that the maximum force and the break force were significantly higher in the male alcohol-exposed rats than in the female alcohol-exposed rats. This may be attributed to sexual dimorphism, with males typically have larger, and stronger bones compared to females (Gordon & Gordon, 2020). During puberty in males, periosteal apposition of bone increases the width of bone and endosteal resorption enlarges the medullary cavity. This leads to an increase in cortical thickness as the periosteal apposition is greater than the endosteal resorption. In females, periosteal apposition decreases at an earlier stage with no changes in medullary size, which leads to bone with a smaller total size and medullary size to males. This leads to a similar cortical thickness (Wang & Seeman, 2008). Even though cortical thickness is similar between sexes, the mass of cortical bone is greater in males due to the greater perimeter of the larger bone (Seeman, 2001).

CONCLUSION

The findings of this study indicate that acute binge alcohol exposure to the osseous tissue with a short duration of 7 days in adolescence has a negative effect on trabecular morphometry in female rat mandibles, resulting in a decreased BV/TV and wider trabeculae. However, the dosage and duration of alcohol exposure had no effect on the mandibular trabeculae morphometry in male rats or on the tensile strength.

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BHIKA, A.; PILLAY, D. & IHUNWO, A.O. Examen del impacto del consumo excesivo de alcohol en la morfometría trabecular y la resistencia a la tracción en mandíbulas de ratas adolescentes Sprague Dawley. *Int. J. Morphol., 42(4)*:905-910, 2024.

RESUMEN: El consumo de alcohol entre menores de edad se ha convertido en una importante preocupación pública que tiene un impacto negativo en el crecimiento y desarrollo del esqueleto. La masa ósea máxima se alcanza durante la adolescencia, por lo que el objetivo del estudio fue investigar el efecto del consumo excesivo de alcohol en forma aguda sobre la morfometría trabecular y la resistencia a la tracción de la mandíbula en ratas adolescente Sprague Dawley (SD). El estudio estuvo compuesto por 24 ratas, de 7 semanas de edad, colocadas en el grupo control expuesto al alcohol [n=12 (6 machos y 6 hembras)] y alimentado en parejas [n=12 (6 machos y 6 hembras)]. El tratamiento de los grupos fue el siguiente; al grupo expuesto al alcohol y al control alimentado en parejas se les administró una dosis única diaria de 3 g/kg de alcohol al 20 % 3 días a la semana (días alternos) durante 7 días y una dosis equivalente calórica de maltosa dextrina mediante sonda oral, respectivamente. Los animales fueron sacrificados el día 7 mediante inyección de pentobarbital. Las mandíbulas se recolectaron y se escanearon utilizando un escáner 3D-microCT Nikon XTH 255L (Nikon Metrology, Lovaina, Bélgica), y las pruebas biomecánicas se realizaron utilizando una máquina de prueba de resistencia a la tracción universal Shimadzu (China). Después del escaneo y la reconstrucción, la morfometría trabecular se evaluó utilizando el software Volume Graphics

Studio®. Se utilizó una prueba de flexión de 3 puntos para evaluar la resistencia a la tracción del hueso. Los hallazgos de nuestro estudio mostraron cambios en algunos parámetros trabeculares en el grupo de hembras expuestas al alcohol, mientras que los grupos de machos no se vieron afectados. No se observaron cambios en la resistencia a la tracción al comparar los grupos control de machos alimentados en parejas y los grupos expuestos al alcohol y al comparar los grupos control de las hembras alimentadas en parejas y los grupos expuestos al alcohol. Se observaron diferencias trabeculares y de resistencia a la tracción entre los sexos al comparar los grupos control de los machos alimentados en parejas y expuestos al alcohol con los grupos de control de hembras alimentadas en parejas y expuestas al alcohol. Estos hallazgos sugieren que el consumo excesivo de alcohol tiene efectos perjudiciales sobre la microarquitectura ósea en ratas hembras expuestas al alcohol y que se observan diferencias entre los sexos.

PALABRAS CLAVE: Adolescente; Consumo compulsivo agudo; Alcohol; Biomecánica; Mandíbula.

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