

The Effects of Acute and Chronic Binge Drinking Followed by Alcohol Abstinence on Mandibular Morphometry in Female Adolescent Sprague Dawley Rats

Efectos del Consumo Excesivo de Alcohol Agudo y Crónico Seguido de la Abstinencia de Alcohol en la Morfometría Mandibular en Ratas Sprague Dawley Adolescentes Hembras

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SUMMARY: Alcohol binge drinking among adolescents is a cause for concern in South Africa and worldwide due to its harmful effects on the growth and development of the adolescent skeleton. This study aimed to investigate the impact of acute and chronic binge drinking on mandibular osteometry parameters in adolescent Sprague Dawley (SD) rats and to evaluate if recovery of the mandible is possible once alcohol is removed from the diet. 36 female SD rats, aged 7 weeks, were divided into alcohol-exposed and pair-fed control groups for acute binge, chronic binge, or alcohol abstinence models. The alcohol-exposed group received 3 g/kg of 20 % alcohol 3 days a week for 7 or 28 days, followed by a 30-day abstinence period in the abstinence model. The pair-fed control groups received a caloric equivalent dose of maltose dextrin. The osteometry measurements were taken from harvested mandibles for analysis. The study found differences in height measurements among all three models. In the acute binge alcohol model, the mandibular ramus height increased significantly in the alcohol-exposed group than the pair-fed control. In the chronic binge alcohol model, the height of the coronoid showed significant increases in the alcohol-exposed group. Additionally, in the chronic binge abstinent model, distances between specific points were greater in the alcohol-exposed animals compared to the pair-fed controls. This research reveals that binge drinking can significantly impact the growth and development of the mandible, both in the short term and over the long term.

KEY WORDS: Adolescent; Binge Alcohol; Bone; Mandible; Morphometry.

INTRODUCTION

Binge drinking in adolescents is common in South Africa and globally (Morojele & Ramsoomar, 2016), having dire consequences on the growth and development of the adolescent skeleton. Binge drinking in adolescents is a risk factor for long-term progression to excessive chronic alcohol consumption in adulthood and is also a risk factor for alcohol-related problems such as motor vehicle accidents (MVAs) and deaths, interpersonal violence, and crime (Morojele & Ramsoomar, 2016; Kuntsche *et al.*, 2017).

Binge drinking is defined as consuming ≥ 5 drinks for males and ≥ 4 drinks for females on one occasion or during one session of drinking, or consuming ≥ 60 g of pure alcohol at least once per month (Morojele & Ramsoomar, 2016).

Adolescence is a period between ages 10 and 19 years during which the skeleton grows and develops rapidly. This era of life accounts for about 90 % of skeletal development and peak bone mass attainment. Therefore, adolescents are considered nutritionally vulnerable because various factors might impact their development and growth (Weaver, 2002; Das *et al.*, 2017; Monge, 2018). Alcohol is a known toxic tetragon that causes deleterious effects on the skeleton (Callaci *et al.*, 2006; Sampson *et al.*, 1999) and also increases the risk of fractures with delayed healing (Sampson *et al.*, 1996; Hogan *et al.*, 1997; Sampson, 1997; Chakkalakal, 2005; LaBrie *et al.*, 2018).

However, adolescents' skeletal systems may be able to heal from the harm inferred by the alcohol if it is removed

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from the diet since this age group is still growing and developing. Lauing *et al.* (2008) discovered that after binge alcohol consumption, the tibia recovered from deficiencies in bone mass, but the vertebrae did not. Literature indicates that alcohol has varying effects on the length of long bones. Alcohol consumption caused a decrease in tibial length in young rats exposed to chronic alcohol (Rosa *et al.*, 2019). Sampson *et al.* (1996), reported that alcohol caused a decrease in the length of the femur after different durations of exposure (2, 4, 6, 8, and 10 weeks) in rats exposed to an alcohol-liquid diet. Hogan *et al.* (1999), however, found that alcohol did not affect the bone length at different time intervals in a chronic alcohol consumption model of a liquid alcohol diet. Alcohol administered via oral gavage in a binge model caused an increase in the length of the femur and tibia (Sampson *et al.*, 1999). The range of results observed, are all likely due to differences in study design, model, and duration of alcohol exposure. To date there have been numerous studies on the effects of alcohol on long bones (Sampson *et al.*, 1996; Hogan *et al.*, 1999; Sampson *et al.*, 1999) with limited research on craniofacial bones such as the mandible.

The mandible is a unique bone that undergoes both endochondral and intramembranous ossification. The condylar process of the mandible is the only part of this osseous tissue that develops through endochondral ossification (Mizoguchi *et al.*, 2013). The rest of the mandible develops from intramembranous ossification. The overall size of the mandible increases significantly between the ages of 11 and 17, with a significant increase in condylar height during this adolescent period (Smartt Jr. *et al.*, 2005). Intramembranous ossification typically occurs earlier in prenatal development. During the initial stages of mandibular development in the rat embryo, mesenchymal cells condense and differentiate directly into osteoblasts. This process starts around embryonic day 13-14. These osteoblasts then lay down bone matrix directly, forming the mandibular bone proper (mandibular body) (Chen *et al.*, 2015).

Endochondral ossification occurs later in prenatal development and is primarily associated with the development of the condylar process of the mandible. This process starts with the formation of a cartilage template within the condylar region. The condylar cartilage begins to develop from mesenchymal cells around embryonic day 16-17 in rats (Chen *et al.*, 2015). Chondrocytes within this cartilage model undergo proliferation and hypertrophy, leading to the formation of primary growth centers in the condylar secondary cartilage (CSC), the angular synchondrosis (AS), and the mandibular midline synchondrosis (MS). The AS and MS fuse and the mandibular condyle reverts to a secondary growth site. Blood

vessels and osteoblasts invade the calcified cartilage, replacing it with bone tissue. This process continues postnatally and throughout early life as the condylar process undergoes growth and remodelling (Chen *et al.*, 2015). The development of the mandibular condyle not only leads to an increase in the size of the mandible but also results in the anteroinferior displacement (transposition) of the mandible.

It is established that alcohol perturbs bones formed by endochondral ossification (Mizoguchi *et al.*, 2013). However, there is still a gap in the literature pertaining to how alcohol affects the mandible which is formed by both types of ossification.

Thus, this study investigated the effects of acute and chronic binge drinking followed by a period of alcohol abstinence on the osteometric morphometry of the adolescent Sprague Dawley rat mandible.

MATERIAL AND METHOD

Study animals. Thirty-six female rats aged seven weeks, were placed into either the alcohol-exposed (n = 6) or pair-fed control group (n = 6) per model, 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) and a 12-hour light/dark cycle. The rats were housed in pairs 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. The study received ethical approval from the Animal Research Ethics Committee, University of the Witwatersrand (AREC 2020/11/02C).

Model allocation:

Acute binge alcohol model: These animals were exposed to alcohol or maltose dextrin for 7 days. **Chronic binge alcohol model:** These animals were exposed to alcohol or maltose dextrin for 28 days. **Binge alcohol abstinent model:** These animals were exposed to alcohol or maltose dextrin for 28 days which was then followed by a 30-day abstinent period to allow time for possible recovery from the alcohol exposure.

Alcohol-exposed rats. 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 3 days per week (on every alternate day). No alcohol was administered during the remaining 4 days of the week. Blood alcohol concentrations (BACs) were tested an hour after exposure by drawing blood using the

exposed: mean = 0.86 g $\pm$ 0.03; (p = 0.215); chronic binge alcohol model: pair-fed control: mean = 0.94 g $\pm$ 0.04, alcohol-exposed: mean = 0.90 g $\pm$ 0.08 (p = 0.182); chronic alcohol abstinent model: pair-fed control: mean = 1.15 g $\pm$ 0.10, alcohol-exposed: mean = 1.16 g $\pm$ 0.03 (p = 0.383).

Mandibular length. No significant differences were identified between the pair-fed control and alcohol-exposed groups in mandibular length measurements in all three models (Table III).

Mandibular height

Acute binge alcohol model. Significant differences were exhibited in the Cd-Go measurement (mandibular ramus height which is the distance between the condyle (Cd) and the gonion (Go)) where the alcohol-exposed group (mean = 7.49 mm $\pm$ 0.15) had a significantly greater Cd-Go than the pair-fed control group (mean = 7.23 mm $\pm$ 0.17) (p = 0.011). All other height measurements showed no significant differences between the groups (Table IV).

Chronic binge alcohol model. Pair-fed control and alcohol-exposed groups showed significant differences in the Cr-Ag measurement (height of the coronoid which is the distance between the coronoid (Cr) and ante-gonion (Ag)) where the alcohol-exposed group (mean = 12.97 mm $\pm$ 0.27) had a significantly greater Cr-Ag than the pair-fed control group (mean = 12.71 mm $\pm$ 0.11) (p = 0.027) (Table IV).

Chronic binge abstinent model. Significant differences were observed between the pair-fed control and alcohol-exposed groups, where greater MF-Ali (distance between the mental foramen (MF) and the deepest point of the outer margin of the bone (Ali)), and greater MF-M1 (distance between the mental foramen (MF) and the highest point of mesiobuccal cusp of the lower 1st molar (M1)) measurements were exhibited in the alcohol-exposed group (MF-Ali: mean = 2.88 mm $\pm$ 0.03; MF-M1: mean = 4.50 mm $\pm$ 0.09) when compared to the pair-fed control (MF-Ali: mean = 2.756 mm $\pm$ 0.08; MF-M1: mean = 4.31 mm $\pm$ 0.12) (p = 0.004 and p = 0.006, respectively) (Table IV).

Table III. Mandibular length in all three models.

Group		Acute				Chronic			Chronic abstinent		
		N	Mean	SD	P-value	Mean	SD	P-value	Mean	SD	P-value
Cd-Id	Pair-fed	6	25.04	0.31	0.181	25.42	0.27	0.305	26.39	0.56	0.336
	Alcohol	6	25.24	0.41		25.24	0.78		26.28	0.53	
Go-Id	Pair-fed	6	24.73	0.29	0.302	25.04	0.27	0.362	26.19	0.57	0.273
	Alcohol	6	24.84	0.42		24.92	0.78		26.01	0.47	
Al-Id^l	Pair-fed	6	8.24	0.23	0.390	8.46	0.14	0.070	8.92	0.26	0.415
	Alcohol	6	8.19	0.23		8.28	0.23		8.96	0.35	
M3-Id^l	Pair-fed	6	14.0	0.12	0.449	14.39	0.16	0.232	14.98	0.30	0.097
	Alcohol	6	14.07	0.29		14.27	0.36		14.73	0.31	

Table IV: Mandibular height in all three models.

		Acute				Chronic			Chronic abstinent		
		N	Mean	SD	P-value	Mean	SD	P-value	Mean	SD	P-value
Cd-Go	Pair-fed	6	7.23	0.17	0.011*	7.62	0.11	0.310	8.37	0.21	0.172
	Alcohol	6	7.49	0.15		7.49	0.23		8.51	0.29	
Cd-Ag	Pair-fed	6	11.12	0.32	0.106	11.34	0.15	0.208	12.21	0.49	0.358
	Alcohol	6	11.34	0.30		11.47	0.32		12.30	0.38	
Cr-Ag	Pair-fed	6	12.87	0.31	0.255	12.71	0.11	0.027*	13.63	0.25	0.135
	Alcohol	6	12.71	0.46		12.97	0.27		13.82	0.30	
Al^l-Me	Pair-fed	6	4.09	4.05	0.295	4.11	0.10	0.460	4.60	0.07	0.093
	Alcohol	6	0.15	0.12		4.10	0.09		4.52	0.11	
MF-Al^l	Pair-fed	6	1.84	0.07	0.345	2.04	0.07	0.222	2.76	0.08	0.004*
	Alcohol	6	1.87	0.12		2.02	0.05		2.88	0.03	
MF-M1	Pair-fed	6	3.87	0.11	0.420	3.98	0.07	0.275	4.31	0.12	0.006*
	Alcohol	6	3.89	0.16		4.01	0.11		4.49	0.09	

DISCUSSION

The current study was to understand the effects of acute and chronic binge drinking followed by a period of alcohol abstinence on the osteometric parameters of the mandible in the adolescent Sprague Dawley rat. Altered dimensions were observed in the alcohol-exposed groups in all three models.

There were no differences in weight between the paired control and alcohol-exposed groups. The results of our study can be corroborated with the study that Sampson *et al.* (1999), conducted where they analyzed the effect of binge drinking on bone metabolism in a young actively growing rat model. They observed a decrease in the weight of the femur; however, no effect was exhibited on the tibia. The lack of changes in the bone weight could thus be bone-specific which may also explain why no differences were identified in our study.

The mandibular ramus height which is the distance between the condyle (Cd) and the gonion (Go) was greater in the alcohol-exposed group in the acute binge alcohol model. In the chronic binge alcohol model, the height of the coronoid which is the distance between the coronoid (Cr) and antegonion (Ag) was significantly greater in the alcohol-exposed group. Significant differences were also observed in the chronic binge abstinent model in the distance between the mental foramen (MF) and the deepest point of the outer margin of the bone (All), and in the distance between the mental foramen (MF) and the highest point of the mesiobuccal cusp of the lower 1st molar (M1). All four of these measurements represent mandibular height. Cd-Go and Cr-Ag are height measurements displayed in the posterior mandible, and MF-All and MF-M1 are height measurements exhibited in the anterior mandible. Sampson *et al.* (1999) detected a similar effect in long bones, where rats exposed to a binge alcohol model via oral gavage also displayed an increase in bone size and attributed their findings to the following two reasons: Firstly, binge drinking in their study did not bring about BAC levels that were as high as those previously identified in chronic drinking models, which would modulate the effects of alcohol on the skeleton. Secondly, the mode of administration was oral gavage, whereas the chronic studies that they had previously conducted exposed animals to alcohol via feeding tubes with a liquid alcohol diet.

The increase in mandibular height observed in our study is likely due to one of two reasons, or a combination of both. The first is the study design (mode of alcohol administration and duration of exposure), and the second is the process of the development of the mandible. In the prenatal development of the rat mandible, both intramembranous and endochondral ossification processes occur at different stages.

Intramembranous ossification typically occurs earlier in prenatal development forming the mandibular bone proper (mandibular body) (Chen *et al.*, 2015). Endochondral ossification occurs later in prenatal development and is primarily associated with the development of the condylar process of the mandible. The length of the body of the mandible increases in a rectilinear direction, whereas the condylar process exhibits growth in various directions from anterosuperior to posterior (Mizoguchi *et al.*, 2013). This diverse growth pattern enables a wide range of growth and shapes of the mandible.

The growth of the condyle is closely related to the transposition of the mandible, which will in turn influence the angle of the mandible, thus the maxillary mandibular relationship. In individuals with low angles (short face, brachyfacial pattern, and deep bite), the mandibular growth is characterised by anterosuperior growth of the condyle. In individuals with high angles (long face, dolichofacial pattern, and skeletal open bite), posterosuperior growth of the condyle is observed (Mizoguchi *et al.*, 2013). Disturbances in growth can thus have a varying range of effects on the length and height of the mandible. In the case of juvenile rheumatoid arthritis, the posterior margin of the ramus had bone apposition and grew in a posterior direction (Mizoguchi *et al.*, 2013). In a study where the effect of alcohol was examined in a fetal alcohol model, measurements of mandibular length indicated that alcohol primarily affected the posterior region of the mandible. This teratogenic effect could be attributed to alcohol's impact on the mitotic activity of the condyle, which plays a significant role in mandibular growth (Hernandez, 1990).

The processes of development of the mandible along with transposition of the mandible could indicate that exposure to alcohol in the acute binge and chronic binge alcohol models in our study showed a similar growth pattern as was displayed in the cases above, with more bone apposition in the posterosuperior direction, which could lead to a mandible with a high angle, with complications such as malocclusion and an open bite. In the binge alcohol abstinent model, increased height in the posterior mandible corrected itself, however, the increase in height in the anterior mandible (anterosuperior growth pattern) may also be due to initial disturbances in the growth of the condyle due to alcohol exposure or due to compensation of the initial posterosuperior growth. The mandibular condyle in rat's fuses at approximately 14 weeks of age (Zoetis *et al.*, 2003). The rats in our study were younger than this at termination which means that the condyle was not yet fused thus allowing alcohol to have a negative effect on both the endochondral and intramembranous growth of the mandible. No literature is available to validate these findings.

CONCLUSION

In conclusion, our findings of an increase in height in mandibular parameters in all three alcohol models (acute binge, chronic binge, and binge alcohol abstinent models) are most likely due to alcohol impacting the growth of the mandibular condyle leading to alterations in the direction of growth of the mandible, with residual effects even after alcohol has been removed from the diet.

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BHIKA, A.; IHUNWO, A.O. & PILLAY, D. Efectos del consumo excesivo de alcohol agudo y crónico seguido de la abstinencia de alcohol en la morfometría mandibular en ratas Sprague Dawley adolescentes hembras. *Int. J. Morphol.*, 43(1):41-46, 2025.

RESUMEN: El consumo excesivo de alcohol entre adolescentes es motivo de preocupación en Sudáfrica y en todo el mundo debido a los efectos nocivos en el crecimiento y desarrollo del esqueleto en ellos. Este estudio tuvo como objetivo investigar el impacto del consumo excesivo de alcohol agudo y crónico en los parámetros de osteometría mandibular en ratas Sprague Dawley (SD) adolescentes y evaluar si es posible la recuperación de la mandíbula una vez que se elimina el alcohol de la dieta. Se dividieron 36 ratas SD hembra, de 7 semanas de edad, en grupos control expuestos al alcohol y alimentados por pares para modelos de consumo excesivo de alcohol agudo, consumo excesivo de alcohol crónico o abstinencia de alcohol. El grupo expuesto al alcohol recibió 3 g/kg de alcohol al 20 % 3 días a la semana durante 7 o 28 días, seguido de un período de abstinencia de 30 días en el modelo de abstinencia. Los grupos control alimentados en pareja recibieron una dosis equivalente calórica de maltosa dextrina. Las mediciones de osteometría se tomaron de mandíbulas extraídas para su análisis. El estudio encontró diferencias en las mediciones de altura entre los tres modelos. En el modelo de embriaguez aguda, la altura de la rama mandibular aumentó significativamente en el grupo expuesto al alcohol que en el control alimentado en pareja. En el modelo de embriaguez crónica, la altura del proceso coronoide mostró aumentos significativos en el grupo expuesto al alcohol. Además, en el modelo de abstinencia crónica, las distancias entre puntos específicos fueron mayores en los animales expuestos al alcohol en comparación con los controles alimentados en pareja. Esta investigación revela que el consumo excesivo de alcohol puede afectar significativamente el crecimiento y el desarrollo de la mandíbula, tanto a corto como a largo plazo.

PALABRAS CLAVE: Adolescente; Embriaguez alcohólica; Hueso; Mandíbula; Morfometría.

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