*Int. J. Morphol.,*
***44(4)***:1461-1468, 2026.

# The Impact of Binge Alcohol Consumption on the Developing Adolescent Sprague Dawley Rat Humerus

## Impacto del Consumo Episódico Excesivo de Alcohol en el Húmero de Ratas Sprague Dawley Adolescentes en Desarrollo

**Rethabile Rakgoathe[1]; Illke Malungo[1] & Akaashni Bhika[1]**

**RAKGOATHE, R.; MALUNGO, I. & BHIKA, A.** The impact of binge alcohol consumption on the developing adolescent Sprague Dawley rat humerus. *Int. J. Morphol., 44(4)*:1461-1468, 2026.

**SUMMARY:** Adolescence is a critical period for skeletal development during which peak bone mass is acquired. Binge drinking, a prevalent form of alcohol misuse among adolescents, has been associated with impaired bone growth and reduced skeletal integrity. Although alcohol-induced skeletal alterations have been widely studied in weight-bearing bones such as the femur and tibia, its effects on the humerus and the potential for skeletal recovery following abstinence remain poorly understood. This study investigated the effects of binge drinking on adolescent humeral structure, strength, and development, along with recovery potential after a period of alcohol abstinence, using a Sprague Dawley rat model. Animals were divided into two experimental groups: Group 1 (four weeks of alcohol exposure) and Group 2 (four weeks of alcohol exposure followed by 30 days of abstinence). Each group consisted of an alcohol-exposed (A) and a pair-fed control (B) subgroup, with 12 rats per group (6 males and 6 females). Alcohol-exposed groups received a 3 g/kg dose of a 20 % alcohol solution three times per week, and pair-fed control groups received an isocaloric equivalent of maltodextrin. Humeral microarchitecture was assessed using micro-computed tomography (3DμCT), biomechanical properties were evaluated using three-point bending, and growth plate proliferation was assessed using Ki-67 immunohistochemistry. Alcohol administration produced blood alcohol concentrations consistent with binge exposure (141–164 mg/dL). After four weeks of exposure, females demonstrated a modest reduction in humeral weight compared with controls ($p = 0.045$). Pronounced sex differences were observed across osteometric parameters, with males exhibiting greater humeral weight, length, and bicondylar breadth ($p < 0.001$). 3DμCT analysis revealed higher bone volume fraction in female distal epiphyses ($p = 0.009$). Following abstinence, alcohol-exposed males exhibited increased trabecular numbers and reduced trabecular separation ($p < 0.05$), while biomechanical testing demonstrated reduced fracture times in female humeri. These findings suggest that adolescent binge drinking may induce persistent, sex-specific changes in humeral microarchitecture and mechanical properties that are not fully reversed following short-term abstinence.

**KEY WORDS: Adolescent; Alcohol; Binge Drinking; Humerus.**

## INTRODUCTION

Binge drinking is the consumption of ≥5 standard alcoholic beverages for men and ≥4 standard alcoholic beverages for women in a single occasion, leading to a blood alcohol concentration of ≥80mg/dL (Gowin *et al*., 2021). This pattern of excessive alcohol consumption is particularly prevalent among adolescents, a developmental stage characterised by rapid skeletal growth and the accumulation of peak bone mass (Weaver, 2002). Peak bone mass attained during adolescence is critical for maintaining skeletal strength and its biomechanical properties for life (Weaver, 2002). Consequently, exposure to harmful factors such as excessive alcohol consumption during this period may have lasting effects on bone health.

Excessive alcohol consumption, such as binge drinking, induces skeletal pathology, and previous studies indicate that skeletal harm can be associated with adolescent binge drinking. In terms of bone strength and structure, various studies have observed direct correlations between adolescent binge drinking and reduced bone density, bone length, bone weight, calcium presence, compressive strength, and rigidity in long bones like the femur and tibia (Sampson *et al*., 1996; Callaci *et al*., 2004). In terms of bone growth and development, literature indicates that alcohol exposure attenuates hormones such as growth hormone (GH), oestrogen, and parathyroid hormone (PTH), which regulate chondrocyte activity within the epiphyseal growth plate in

[1]Department of Anatomical Sciences, School of Biomedical Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.
FUNDING. This study was funded by the National Research Foundation of South Africa; Grant number: TTK210404592026.

Received: 2026-04-23 Accepted: 2026-06-27

long bones (Loud & Gordon, 2006). Reduced activity of these hormones impairs chondrocyte proliferation and alters growth plate morphology, potentially compromising longitudinal bone growth. Importantly, the skeletal effects of alcohol are not uniform, as previous work suggests that the effect of alcohol on the skeleton is observed to be site-specific between bones and within a particular bone itself (Lauing *et al*., 2008).

Sexual dimorphism represents another important factor influencing the skeletal response to alcohol exposure. However, studies investigating binge drinking have focused predominantly on a single sex (Sampson *et al*., 1996; Lauing *et al*., 2008). Physiological differences between males and females influence alcohol metabolism and endocrine regulation. For example, alcohol dehydrogenase (ADH) activity in the gastric mucosa is higher in males than in females, resulting in more rapid inebriation in females (Wilsnack *et al*., 2018). In addition, key hormones involved in skeletal development, such as GH, oestrogen, and PTH, function differently between the sexes, and their dysregulation leads to sex-specific physiological disturbances (Block *et al*., 1993). Alcohol consumption has been associated with reduced oestrogen levels in females, while GH levels decline in both sexes following alcohol exposure, the reduction is more pronounced in females, potentially leading to greater skeletal vulnerability in females (Dees & Skelley, 1990).

Sex differences in skeletal development may further influence the response of long bones to alcohol exposure. Growth plate fusion occurs earlier in females (approximately 14–17 years) than in males (15–19 years) (Kvist *et al*., 2021), and proximal and distal epiphyseal plates do not close simultaneously. In the humerus specifically, proximal growth plates remain open and fuse between 12 and 20 years, whereas distal plates typically close earlier between 11 and 19 years (Zoetis *et al*., 2003). This asynchronous closure contributes to the higher incidence of proximal humeral fractures (Lill & Josten, 2000). Additionally, structural differences in humeral width and length are established before puberty, with males exhibiting a greater periosteal diameter than females (Clark *et al*., 2007). Because the periosteum plays a key role in bone repair, the wider periosteal layer in males may allow better compensation for alcohol-induced damage. Consequently, the detrimental skeletal effects of binge drinking may be more pronounced in females, while males may exhibit relatively higher trabecular number and bone mineral density (Clark *et al*., 2007).

Although previous studies have extensively investigated the femur and tibia, the humerus remains relatively understudied in the context of adolescent binge drinking. Furthermore, it is also unclear whether the adolescent skeleton can fully recover following binge drinking once abstinence is practiced. Therefore, this study examined the impact of binge drinking on bone structure (both external and internal), mechanical strength, and development of the adolescent humerus. Moreover, this study explored the recovery potential of the humerus following a period of alcohol abstinence. Addressing these gaps is important for understanding the potential long-term consequences of adolescent alcohol misuse on upper limb skeletal health.

## MATERIAL AND METHOD

### Study animals

Ethical clearance for this study was obtained from the Animal Research Ethics Committee (AREC) of the University of the Witwatersrand (Clearance number: 2020/11/02/C). Animal care and experimental procedures were performed in accordance with institutional guidelines for the ethical use of laboratory animals and adhered to national standards for animal welfare. The study included 48 adolescent Sprague Dawley rats (24 males and 24 females) aged 7 weeks (49 postnatal days) with body weights ranging from 175 - 199 g. All the study animals were housed in 430mm (length) x 220mm (width) x 200mm (height) cages, where two rats of the same sex were paired together in each cage. They were maintained under an environment free of most pathogens, which was temperature-controlled (26-28°C), and consisted of a 12-hour light/dark cycle to regulate their circadian rhythm. Furthermore, the animals received a standard diet of rat food and tap water *ad libitum*.

**Group allocation and administration**. Animals were randomly allocated into four experimental groups (n = 12 per group, 6 males and 6 females):

**Group 1A: Alcohol-exposed group (4 weeks):** These animals were exposed to alcohol for 4 weeks, adopting a chronic binge alcohol model. Alcohol was administered once daily via oral gavage as a 20 % (v/v) alcohol solution at a dose of 3g/kg. Alcohol was administered three days per week on alternate days, with no alcohol given during the remaining four days.

Blood alcohol concentrations (BACs) were measured one-hour post-administration by collecting blood via the tail-prick method. Samples were stored in heparinized microcapillary tubes (Marienfeld). BACs were determined using a colorimetric alcohol assay kit (Sigma-Aldrich), with absorbance readings obtained at 630 nm.

**Group 1B: Pair-fed control group (4 weeks):**As a caloric control, a pair-fed rat was individually matched to each alcohol-fed rat based on initial body weight. The pair-fed group received an isocaloric equivalent of maltodextrin (Sigma-Aldrich), which was administered via oral gavage on the same days as Group 1A.

**Group 2A: Binge-alcohol recovery group:** To evaluate bone recovery following the effects of binge drinking, an additional group of rats was exposed to alcohol for 28 days (4 weeks) using the same protocol as Group 1A. Following this exposure period, the animals were maintained alcohol-free for an additional 30-days prior to euthanasia (Lauing *et al*., 2008).

**Group 2B: Binge-alcohol recovery pair-fed control group:** These animals received maltodextrin for 28 days according to the pair-fed control protocol of Group 1B. They were subsequently maintained for an additional 30 days without maltodextrin prior to euthanasia.

**Termination and skeletal harvesting**

The animals were euthanized on day 28 (Groups 1A and 1B) and day 58 (Groups 2A and 2B) by pentobarbital intraperitoneal injection. The humeri were harvested and stored in 10 % buffered formalin for further fixation and processing.

**Three-Dimensional micro-computed tomography scanning (3D-μCT)**

Bone microarchitecture was assessed using a Nikon XTH 225/320 LC X-ray microtomograph. Scanning parameters include X-ray voltage of 80 kV, X-ray current of 100μA, a scanning resolution of 22 μm, and a scan duration of 8 min. Analysis was performed using VG Studio Max® 3.2 software to evaluate bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), and trabecular separation (Tb.Sp). The region of interest (ROI) was defined as the trabecular bone within the epiphysis immediately proximal to the epiphyseal growth plate.

**Biomechanical testing**

Mechanical properties of the left humerus were assessed using a Shimadzu three-point bending machine. Bones were positioned on two supports spaced 15mm apart, and a mediolateral force was applied to the midshaft of the humerus. Load-displacement curves were recorded at a constant speed of 5mm/min until bone failure occurred for each specimen. The following parameters were tested: maximum force, which is the force necessary to deform a bone; displacement, which is the vertical deviation from the horizontal plane necessary to fracture a bone; time to fracture, which is the duration of which a bone takes to resist the force until failure; and break force, which is the force necessary to fracture a bone.

**Tissue processing for immunohistochemical techniques**

The right humeri were used for immunohistochemistry (IHC). The humeri were fixed in 10 % buffered formalin for at least 14 days and subsequently decalcified in 14 % (w/v) ethylenediaminetetraacetic acid (EDTA) solution (pH 7.4; Sigma-Aldrich, USA) for 21 days at 45 °C in an incubator with continuous gentle shaking. The specimens were examined to see if they were completely decalcified using the Aribex NOMAD portable X-ray system. Calcium presence gives rise to a white (radio-opaque) appearance visualised during radiography. Decalcification, however, causes the loss of this white appearance, and the bone instead appears transparent (radiolucent) (Choi *et al*., 2015). Visualising a radiolucent appearance of the humeri on the X-ray system therefore confirmed decalcification.

Following decalcification, the humeri were sectioned into proximal (humeral head) and distal portions, and these portions were left in 10 % buffered formalin for 24 h before processing. The samples were then processed through ascending grades of alcohol overnight using an automatic tissue processor (Citadel 2000, Thermo Fisher Scientific, USA), before embedding in paraffin wax. Serial sections were then cut with the Leica (Leica RM2125 RTS; Leica Biosystems, Germany) microtome at 5 μm thickness in a longitudinal plane and mounted onto 3 % silane coated slides. Slides were coated with 3 % silane for adherence of tissues during staining and immunohistochemistry procedures. Every first and second section was alternately immunolabeled with anti-Ki-67 antibody and its negative control, respectively, to evaluate the impact of binge drinking on the proliferation of epiphyseal growth plate proliferative cells.

**Immunohistochemistry for Ki-67**

Immunohistochemical detection of proliferating cells was performed using an anti-Ki-67 primary antibody (NB 500-170; Whitehead Scientific, South Africa). The sections were deparaffinized, rehydrated, and subsequently, immersed in citrate buffer solution (pH=6) kept in a water bath set at a temperature of 60 °C for 2 h to allow for antigen retrieval. Tissue sections were then cooled down to room temperature for 20 min before washing for 5 min in a phosphate buffer solution (PBS; pH=7.4). Endogenous peroxidase was blocked with 1 % hydrogen peroxide ($H_2O_2$), washed with

PBS for 3×5 min, incubated with a goat serum protein block (Ab156024; Abcam, UK) for 20 min, and washed in PBS for 3×5 minutes. The tissue sections were then incubated with primary antibody (anti-Ki-67 in 1:200 dilution) overnight at 4 °C. On the second day, sections were allowed to reach room temperature and washed in PBS for 4×5 min. A biotinylated secondary antibody (biotinylated goat anti-rabbit IgG [H+L]) (ab64256; Abcam, UK) was applied for 10 min and then washed in PBS for 4×5 min. To reduce nonspecific background staining while enhancing antibody binding, sections were incubated with streptavidin horseradish peroxidase for 10 min (ab64269; Abcam, UK) and washed in PBS for 4×5 min. For visualization under a light microscope, sections were incubated with 3-3'di-aminobenzidine (DAB) working solution for 5 min, then rinsed in running tap water for 5 min. The sections were then dehydrated through graded alcohols, cleared in xylene, and mounted in Entellen.

**Photomicrograph acquisition and cell quantification**

Photomicrographs of the stained slides were acquired using a Zeiss Axioscope 2 plus light microscope (Germany) equipped with an Axiocam HRC digital camera. Images were captured at ×10 magnification. The photomicrographs were imported into Fiji ImageJ software, where the entire proliferative zone of the epiphyseal growth plate was analysed. A manual counting method was used for the quantification of Ki-67 positive cells.

**Statistical analysis**

Osteometric measurements, including humeral length, bicondylar breadth, and humeral weights (obtained in triplicate), were organized in Microsoft Excel 2023 (Microsoft Corporation) to calculate mean and standard deviation values. Statistical analyses were performed using Statistical Package for Social Services (SPSS®) version 29 (IBM Co.) Interobserver reliability was assessed, and descriptive statistics, such as mean, standard deviation, median, and mode, were calculated for humeral osteometric parameters, trabecular morphometrics, and chondrocyte counts. Data normality was assessed using the Shapiro-Wilk test. For normally distributed data, independent-samples t-tests were conducted, whereas non-normally distributed data were analysed using median values and the Mann-Whitney U test. A p-value <0.05 was considered statistically significant, at a confidence interval of 95 %.

**RESULTS**

Results are presented according to experimental groups. Group 1 refers to the animals exposed for 4 weeks, and Group 2 refers to the animals exposed for 4 weeks followed by a 30-day alcohol abstinence period.

**Blood Alcohol Concentration**

The mean blood alcohol concentration (BAC) was 141.01 mg/dL (± 22.53) in the alcohol group 1A and 164.05 mg/dL (± 24.70) in the alcohol group 2A. BAC levels in the pair-fed control groups (1B and 2B) were negligible.

**Osteometric Measurements**

**Humeral Weight**

**Group 1:** In females, binge drinking resulted in a modest but significant reduction in humeral weight compared with controls (alcohol-exposed: mean = 0.37 ± 0.02 g; pair-fed control: mean = 0.38 ± 0.01 g; $p = 0.045$). Sex differences were also observed. In the pair-fed control groups, male humeri (0.44 ± 0.15 g) were significantly heavier than female humeri (0.38 ± 0.01 g) ($p = 0.002$). Similarly, binge drinking did not alter this, as male humeri (0.50 ± 0.04 g) remained significantly heavier than female humeri (0.37 ± 0.02 g) in the alcohol-exposed groups ($p < 0.001$).

**Group 2:** Significant sex differences were observed in both treatment groups. In the pair-fed control groups, male humeri (0.61 ± 0.04 g) were significantly heavier than female humeri (0.48 ± 0.04 g) ($p < 0.001$). This observation was consistent in the alcohol-exposed groups, where male humeri (0.61 ± 0.04 g) were heavier than female humeri (0.45 ± 0.02 g) ($p < 0.001$).

**Maximum humeral length**

**Group 1:** The only significant results observed were sex differences. In the pair-fed control groups, male humeri (25.57 ± 8.08 mm) were significantly longer than female humeri (25.25 ± 0.13 mm) ($p < 0.001$). Similarly, in the alcohol-exposed groups, male humeri (27.50 ± 0.76 mm) were significantly longer than female humeri (24.78 ± 0.58 mm) ($p < 0.001$).

**Group 2:** Sex differences remained evident following the abstinence period. Male humeri were significantly longer than female humeri in both pair-fed control (males: 29.96 ± 0.92 mm; females: 26.78 ± 0.48 mm) and alcohol-exposed groups (males: 26.96 ± 0.41 mm, females: 29.62 ± 0.54 mm) ($p < 0.001$ for both comparisons).

**Bicondylar breadth**

**Group 1:** In the pair-fed control group, female humeri (6.82

± 0.11 mm) exhibited slightly greater bicondylar breadth than male humeri (6.76 ± 2.14 mm) ($p = 0.02$). In contrast, within the alcohol-exposed group, male humeri had significantly greater breadths (7.47 ± 0.17 mm) compared with female humeri (6.69 ± 0.28 mm) ($p < 0.001$).

**Group 2:** When comparing the pair-fed control groups between sexes, the males had significantly greater bicondylar breadths (7.55 ± 0.22 mm) than the females (6.93 ± 0.28 mm) ($p < 0.001$). Similarly, in the alcohol-exposed groups, the males had significantly greater breadths (7.49 ± 0.39 mm) than females (6.92 ± 0.21 mm) ($p < 0.001$).

**Trabecular Morphometry (microCT)**

**Group 1:** In the distal epiphyses, females demonstrated significantly greater bone volume fraction (BV/TV) (58.4 ± 0.04 %) compared to males (43.63 ± 0.21 %) ($p = 0.009$).

**Group 2:** In male proximal epiphyses, alcohol exposure followed by abstinence resulted in significant increases in trabecular number (Tb.N), with alcohol-exposed humeri (4.66 ± 0.30 $mm^{-1}$) showing higher Tb.N values than controls (4.24 ± 0.17 $mm^{-1}$) ($p = 0.007$). Alcohol abstinence in males resulted in significantly smaller trabecular spaces (Tb.Sp) in the proximal epiphyses. Alcohol-exposed humeri (0.08 ± 0.02 mm) had reduced Tb.Sp compared to pair-fed control humeri (0.11 ± 0.02 mm) ($p = 0.015$).

**Biomechanical Properties**

**Tensile strength:** Biomechanical testing revealed significant sex differences in humeral mechanical properties (Table I and Table II). In Group 2, females exposed to alcohol followed by a period of abstinence demonstrated significantly reduced fracture times compared with controls.

Table I. Tensile strength of the humerus in male and female rats in Group 1.

| Group 1. Binge alcohol exposed and pair-fed control groups | | | | | |
|---|---|---|---|---|---|
| | | **Maximum force (N)** | **Maximum displacement (mm)** | **Fracture time (s)** | **Break force (N)** |
| **Male** | **PF- control** | 64.27 | 1.87 | 30.03 | 61.1 |
| | **SD** | 5.33 | 5.33 | 33.60 | 10.53 |
| | **Alcohol-exposed** | 61.75 | 1.65 | 32.91 | 60.10 |
| | **SD** | 7.99 | 1.44 | 28.77 | 8.17 |
| **Female** | **PF- control** | 44.83 | 0.78 | 15.62 | 44.72 |
| | **SD** | 3.08 | 0.25 | 4.91 | 3.06 |
| | **Alcohol-exposed** | 42.66 | 0.84 | 16.70 | 41.46 |
| | **SD** | 5.58 | 0.19 | 3.89 | 5.18 |
| **PF vs alcohol** | **p-value (PF vs alcohol male)** | p>0.05 | p>0.05 | p>0.05 | p>0.05 |
| | **p-value (PF vs alcohol female)** | p>0.05 | p>0.05 | p>0.05 | p>0.05 |
| **Male vs female** | **p-value (male vs female PF)** | p<0.001* | p>0.05 | p>0.05 | p=0.002* |
| | **p-value (male vs female alcohol)** | p<0.001* | p>0.05 | p>0.05 | p<0.001* |

Table II. Tensile strength of the humerus in male and female rats in Group 2.

| Group 2. Binge alcohol-exposed and pair-fed control recovery groups | | | | | |
|---|---|---|---|---|---|
| | | **Maximum force (N)** | **Maximum displacement (mm)** | **Fracture time (s)** | **Break force (N)** |
| **Male** | **PF- control** | 87.94 | 1.14 | 22.70 | 87.32 |
| | **SD** | 8.96 | 0.12 | 2.40 | 8.68 |
| | **Alcohol-exposed** | 91.49 | 1.03 | 20.31 | 91.40 |
| | **SD** | 9.28 | 0.22 | 4.45 | 9.32 |
| **Female** | **PF- control** | 60.81 | 0.76 | 26.93 | 60.81 |
| | **SD** | 10.21 | 0.28 | 25.36 | 10.21 |
| | **Alcohol-exposed** | 61.09 | 0.56 | 1.89 | 61.07 |
| | **SD** | 3.22 | 0.08 | 1.49 | 3.22 |
| **PF vs alcohol** | **p-value (PF vs alcohol male)** | p>0.05 | p>0.05 | p>0.05 | p>0.05 |
| | **p-value (PF vs alcohol female)** | p>0.05 | p>0.05 | p=0.009* | p>0.05 |
| **Male vs female** | **p-value (male vs female PF)** | p<0.001* | p=0.007* | p>0.05 | p=0.032* |
| | **p-value (male vs female alcohol)** | p<0.001* | p<0.001* | p<0.001* | p<0.001* |

**Cell proliferation**

**Group 1:** No significant differences in chondrocyte proliferation were observed between alcohol exposed and control animals.

**Group 2:** In the pair-fed control groups, females (422 ± 77.81 cells) had significantly higher numbers of proliferating chondrocytes than males (191 ± 172.32 cells) in the proximal growth plates ($p = 0.009$).

## DISCUSSION

This study investigated the effects of adolescent binge drinking on the humeral structure, mechanical properties, and growth plate cellular activity using a Sprague Dawley rat model. Two experimental conditions were evaluated: a chronic binge consumption model (four weeks of alcohol exposure) and a binge-alcohol recovery model, which incorporated a 30-day abstinence period following alcohol exposure. Overall, the findings demonstrate that adolescent binge drinking induces sex-dependent alterations in humeral morphology, trabecular microarchitecture, and biomechanical integrity, with evidence of incomplete skeletal recovery following abstinence.

In females, alcohol exposure during the chronic binge phase resulted in reduced humeral weight compared with pair-fed controls. It has been previously reported that alcohol may disturb the bone remodelling process in females as it affects oestrogen functioning (Turner & Sibonga, 2001). Oestrogen plays a role in maintaining the life span of bone-forming cells, such as osteoblasts, and reducing the life span of bone-resorbing cells, which are osteoclasts. Moreover, alcohol consumption has been linked to decreased oestrogen levels in females (Turner & Sibonga, 2001). Consequently, when alcohol is consumed in young females, oestrogen levels have been observed to decline, and the life span of osteoblasts decreases while that of osteoclasts increases, encouraging bone resorption, which can lead to decreased bone mass (Turner *et al*., 1994). This could underlie why reduced humeral weights in females were observed. In the binge-alcohol recovery model, biomechanical testing revealed that alcohol-exposed female humeri were significantly easier to fracture even after a period of alcohol abstinence. Alcohol may encourage bone resorption through decreased oestrogen levels, which reduces bone mass accrual. This may underlie the increased fracture risk associated with binge drinking (Turner & Sibonga, 2001). Block *et al*. (1993), reported reduced oestrogen levels two weeks after alcohol exposure in adolescent females after moderate alcohol consumption. The observed result in group 2 females suggests that binge drinking, like moderate alcohol exposure, may reduce oestrogen levels, with effects that persist over time (Block *et al*., 1993).

In contrast, male humeri demonstrated changes in trabecular morphometry, following the abstinence period, with significantly increased trabecular number (Tb.N) and decreased trabecular spacing (Tb.Sp) observed in the proximal epiphyses of group 2 humeri. Heavy alcohol consumption, such as binge drinking, correlates with reduced levels of testosterone in males as alcohol encourages the conversion of testosterone to oestrogen through a process termed aromatisation, which has also been observed in rat models (Gordon *et al*., 1978). During aromatisation, testosterone and its precursor androstenedione are metabolised into oestrogens such as oestradiol and oestrone, respectively; hence, increased oestrogen levels may be detected in binge-drinking males (Gordon *et al*., 1978). Increased oestrogen in males is associated with enhanced bone cell production, such as osteoblasts (Gordon *et al*., 1978). The observations of Gordon *et al*. (1978), support the current findings regarding the observed changes and are suggestive of increasingly robust bone structure as a result of abstinence post an alcohol exposure period.

Regarding the sex differences observed, the findings demonstrated that sexual dimorphism plays a significant role in how males and females respond to alcohol exposure, as male and female animals exhibited distinct differences in response to binge drinking.

In terms of osteometric measurements, males generally displayed greater weights, lengths, and bicondylar breadths compared to females in both study groups. Previous research by Clar7 *et al*. (2006), supports these findings, noting that sex differences in humeral morphology are established before puberty, with males exhibiting larger periosteal diameters resulting in wider and heavier humeri. In terms of trabecular morphometry, females displayed reduced bone volume (BV/TV) and trabecular number (Tb.N) along with greater thickness (Tb.Th) and spacing (Tb.Sp), in the proximal regions of the alcohol-exposed group than the males. Reduced bone volume (BV/TV) and trabecular number (Tb.N) could be indicative of decreased bone formation (Wezeman *et al*., 2007), and the increased trabecular spacing (Tb.Sp) could point to greater bone porosity. Reduced oestrogen levels associated with alcohol consumption in females may underlie these findings (Turner & Sibonga, 2001).

In males, however, moderate alcohol exposure not only leads to altered testosterone levels but also increased oestrogen levels through the aromatization of androgens, promoting bone growth (Purohit, 2000). The dosage of

alcohol (20 % (v/v) may have been moderate for males, as binge drinking is defined differently between sexes, thus supporting humeral growth and recovery (Gowin *et al*., 2021). Male humeri demonstrated greater tensile strength, likely attributed to a larger periosteal diameter influenced by higher testosterone levels that promote periosteal expansion (Seeman, 2003; Clark *et al*., 2007). In females, oestrogen limits periosteal expansion, leading to reduced periosteal diameter compared to males (Seeman, 2003). Since testosterone exerts its effects more in males than females during adolescence and oestrogen is a limiting factor in terms of periosteal diameter, this may underlie the tensile strength disparity observed between the sexes (Li & Fennessy, 2021). During biomechanical testing, female humeri exhibited significantly lower resistance to fractures than male humeri, which correlates with the reduced BV/TV observed during Micro-CT analysis. These results may suggest a potential reduction of humeral strength in females following binge drinking, which might not fully recover during periods of abstinence and could possibly be associated with an increased risk of fracture. This finding aligns with White (2020), who reported that female tissues are more susceptible to the long-term impacts of alcohol.

The IHC analysis using anti-Ki-67 antibodies highlighted the differential responses to alcohol between sexes. In the chronic binge consumption model, female humeri displayed reduced chondrocyte numbers as a consequence of alcohol exposure, while male humeri exhibited an increase in chondrocyte numbers. However, in the binge alcohol recovery model, females had higher chondrocyte numbers than males, even though this difference was non-significant. This aligns with findings from Kalbfleisch *et al*. (1963), and Laitinen *et al*. (1991), who reported hypercalcemia, hypercalciuria, and hypoparathyroidism in males resulting from excessive drinking, which therefore could extend to binge drinking. These conditions stimulate increased PTH release to promote bone formation as a compensatory mechanism for the bone damage.

## CONCLUSION

In conclusion, adolescent binge drinking disrupts the development of the adolescent humerus in a sex-dependent manner. Males appear more resilient, with evidence of structural recovery following abstinence, whereas females exhibit persistent deficits in bone mass and strength. These findings highlight the critical influence of sexual dimorphism on skeletal responses to alcohol and underscore the heightened long-term fracture risk associated with adolescent binge drinking in females. Further research investigating longer recovery periods and the endocrine mechanisms underlying these changes may provide additional insight into the long-term skeletal consequences of adolescent alcohol exposure.

## ACKNOWLEDGEMENTS

I would like to thank Mrs. H. Ali for her technical assistance. I would also like to thank the NRF (Thuthuka grant: TTK210404592026) for funding and the WARF for their assistance with the animal study.

**RAKGOATHE, R.; MALUNGO, I. & BHIKA, A.** El impacto del consumo episódico excesivo de alcohol en el húmero de ratas Sprague Dawley adolescentes en desarrollo. *Int. J. Morphol., 44(4)*:1461-1468, 2026.

**RESUMEN:** La adolescencia es un período crítico para el desarrollo esquelético, durante el cual se alcanza el pico de masa ósea. El consumo episódico excesivo de alcohol (binge drinking), una forma frecuente de uso indebido de alcohol entre los adolescentes se ha asociado con una alteración del crecimiento óseo y una reducción de la integridad esquelética. Aunque las alteraciones esqueléticas inducidas por el alcohol han sido ampliamente estudiadas en huesos de carga como el fémur y la tibia, sus efectos sobre el húmero y el potencial de recuperación esquelética tras la abstinencia siguen siendo poco conocidos. Este estudio investigó los efectos del consumo episódico excesivo de alcohol en la estructura, resistencia y desarrollo del húmero en adolescentes, así como el potencial de recuperación tras un período de abstinencia, utilizando un modelo de rata Sprague Dawley. Los animales se dividieron en dos grupos experimentales: Grupo 1 (cuatro semanas de exposición al alcohol) y Grupo 2 (cuatro semanas de exposición al alcohol seguidas de 30 días de abstinencia). Cada grupo constaba de un subgrupo expuesto al alcohol (A) y un subgrupo control con alimentación pareada (B), con 12 ratas por grupo (6 machos y 6 hembras). Los grupos expuestos al alcohol recibieron una dosis de 3 g/kg de una solución de alcohol al 20 % tres veces por semana, y los grupos control con alimentación pareada recibieron un equivalente isocalórico de maltodextrina. La microarquitectura humeral se evaluó mediante microtomografía computarizada (3DμCT), las propiedades biomecánicas se analizaron mediante ensayos de flexión en tres puntos y la proliferación en la placa de crecimiento se evaluó mediante inmunohistoquímica para Ki-67. La administración de alcohol produjo concentraciones de alcohol en sangre compatibles con una exposición episódica excesiva (141–164 mg/dL). Tras cuatro semanas de exposición, las hembras mostraron una reducción moderada en el peso del húmero en comparación con los controles ($p = 0,045$). Se observaron diferencias sexuales marcadas en los parámetros osteométricos; los machos presentaron mayor peso, longitud y anchura bicondílea del húmero ($p < 0,001$). El análisis por 3DμCT reveló una mayor fracción de volumen óseo en las epífisis distales de las hembras ($p = 0,009$). Tras un periodo de abstinencia, los machos expuestos al alcohol mostraron un mayor número de trabéculas y una menor separación trabecular ($p < 0,05$), mientras que los ensayos biomecánicos revelaron una reducción en los tiempos de fractura en los húmeros de las hembras. Estos hallazgos sugieren que el

consumo intensivo de alcohol durante la adolescencia puede inducir cambios persistentes y específicos según el sexo en la microarquitectura y las propiedades mecánicas del húmero, los cuales no se revierten completamente tras un periodo breve de abstinencia.

**PALABRAS CLAVE: Adolescente; Alcohol; Consumo intensivo de alcohol; Húmero.**

## REFERENCES

Block, G. D.; Yamamoto, M. E.; Mallick, A. & Styche, A. J. Effects on pubertal hormones by ethanol abuse in adolescents. *Alcohol. Clin. Exp. Res., 17(2)*:505, 1993.

Callaci, J. J.; Juknelis, D.; Patwardhan, A.; Sartori, M.; Frost, N. & Wezeman, F. H. The effects of binge alcohol exposure on bone resorption and biomechanical and structural properties are offset by concurrent bisphosphonate treatment. *Alcohol. Clin. Exp. Res., 28(1)*:182-91, 2004.

Choi, S. E.; Hong, S. W. & Yoon, S. O. Proposal of an appropriate decalcification method of bone marrow biopsy specimens in the era of expanding genetic molecular study. *J. Pathol. Transl. Med., 49(3):*236-42, 2015.

Clark, E. M.; Ness, A. R. & Tobias, J. H. Gender differences in the ratio between humerus width and length are established prior to puberty. *Osteoporos. Int., 18(4)*:463-70, 2007.

Dees, L. & Skelley, C. W. Effects of ethanol during the onset of female puberty. *Neuroendocrinology, 51(1)*:64-9, 1990.

Gordon, G. G.; Southren, A. L. & Lieber, C. S. The effects of alcoholic liver disease and alcohol ingestion on sex hormone levels. *Alcohol. Clin. Exp. Res., 2(3)*:259-63, 1978.

Gowin, J. L.; Sloan, M. E.; Morris, J. K.; Schwandt, M. L.; Diazgranados, N. & Ramchandani, V. A. Characteristics associated with high-intensity binge drinking in alcohol use disorder. *Front. Psychol., 12:*750395, 2021.

Kalbfleisch, J. M.; Lindeman, R. D.; Ginn, H. E. & Smith, W. O. Effects of ethanol administration on urinary excretion of magnesium and other electrolytes in alcoholic and normal subjects. *J. Clin. Invest., 42(9)*:1471-5, 1963.

Kvist, O.; Dallora, A. L.; Nilsson, O.; Anderberg, P.; Sanmartin Berglund, J.; Flodmark, C. E. & Diaz, S. A cross-sectional magnetic resonance imaging study of factors influencing growth plate closure in adolescents and young adults. *Acta Paediatr., 110(4)*:1249-56, 2021.

Laitinen, K.; Lamberg-Allardt, C.; Tunninen, R.; Karonen, S. L.; Tähtelä, R.; Ylikahri, R. & Välimäki, M. Transient hypoparathyroidism during acute alcohol intoxication. *N. Engl. J. Med., 324(11)*:721-7, 1991.

Lauing, K.; Himes, R.; Rachwalski, M.; Strotman, P. & Callaci, J. J. Binge alcohol treatment of adolescent rats followed by alcohol abstinence is associated with site-specific differences in bone loss and incomplete recovery of bone mass and strength. *Alcohol, 42(8)*:649-56, 2008.

Li, C. & Fennessy, P. The periosteum: a simple tissue with many faces, with special reference to the antler-lineage periostea. *Biol. Direct, 16(1)*:17, 2021.

Lill, H. & Josten, C. Proximal and distal humeral fractures in the elderly. *Orthopade, 29(4)*:327-41, 2000.

Loud, K. J. & Gordon, C. M. Adolescent bone health. *Arch. Pediatr. Adolesc. Med., 160(10):*1026-32, 2006.

Purohit, V. Can alcohol promote aromatization of androgens to estrogens? A review. *Alcohol, 22(3)*:123-7, 2000.

Sampson, H. W.; Perks, N.; Champney, T. H. & DeFee, B. Alcohol consumption inhibits bone growth and development in young actively growing rats. *Alcohol. Clin. Exp. Res., 20(8)*:1375-84, 1996.

Seeman, E. Periosteal bone formation—a neglected determinant of bone strength. *N. Engl. J. Med., 349(4)*:320-3, 2003.

Turner, R. T. & Sibonga, J. D. Effects of alcohol use and estrogen on bone. *Alcohol Res. Health, 25(4)*:276-81, 2001.

Turner, R. T.; Evans, G. L. & Wakley, G. K. Reduced chondroclast differentiation results in increased cancellous bone volume in estrogen-treated growing rats. *Endocrinology, 134(1)*:461-6, 1994.

Weaver, C. M. Adolescence: the period of dramatic bone growth. *Endocrine, 17(1)*:43-8, 2002.

Wezeman, F. H.; Juknelis, D.; Himes, R. & Callaci, J. J. Vitamin D and ibandronate prevent cancellous bone loss associated with binge alcohol treatment in male rats. *Bone, 41(4)*:639-45, 2007.

White, A. Gender differences in the epidemiology of alcohol use and related harms in the United *States. Alcohol Res., 40(2)*:01, 2020.

Wilsnack, R. W.; Wilsnack, S. C.; Gmel, G. & Kantor, L. W. Gender differences in binge drinking: prevalence, predictors, and consequences. *Alcohol Res., 39(1)*:57-76, 2018.

Zoetis, T.; Tassinari, M. S.; Bagi, C.; Walthall, K. & Hurtt, M. E. Species comparison of postnatal bone growth and development. *Birth Defects Res. B Dev. Reprod. Toxicol., 68(2)*:86-110, 2003.

Corresponding author:
Akaashni Bhika
Department of Anatomical Sciences
School of Biomedical Science
Faculty of Health Sciences
University of the Witwatersrand
7 York Road, Parktown
Johannesburg 2193
South Africa

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