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Research article

Tensile strength and trabecular parameter disturbances at 12 weeks postnatally in Sprague Dawley rats exposed to gestational alcohol

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ABSTRACT

Gestational alcohol exposure impairs bone growth and may cause an increase in osteoporosis and fracture risk later in life. The study aimed to investigate how intrauterine alcohol exposure would impede trabecular morphometric parameters and tensile strength in postnatal 12-week-old rats. Time-mated (n=10) pregnant rats were assigned to an ethanol experimental group (n=5) and a saline control group treated with 0.015ml/g of 25.2% ethanol or 0.9% saline for the initial 19 days of pregnancy through oral gavage, respectively. Two pups from each dam were used, and terminated when aged 12-weeks, and ten paired humerus bones and femora obtained per group. The bones were scanned at 20µm resolution. Bone length, the bone volume to total volume ratio (BV/TV), trabecular thickness (TbTh), number (TbN), and spacing (TbSp) were analysed as well as cortical bone architecture. Then, 3-point bending tests were conducted with a universal tensile tester to obtain the maximum force, displacement, and time, as well as the breaking force. Both the humerus and the femur were shorter in the experimental group (ethanol) with a smaller bone fraction area, fewer trabecular that were more widely spaced in both proximal and distal regions although trabecular thickness was similar in both the ethanol and control (saline) groups. Tensile strength revealed group similarities. Therefore, the effects of gestational alcohol exposure were not severe at 12 weeks of age, this may suggest that there is potential skeletal recovery in adult life following intrauterine alcohol exposure. However, gestational alcohol affected trabecular morphometric parameters at 12 weeks of age.

Keywords: Femur, Humerus, Gestational Alcohol Exposure, Trabeculae, Sprague Dawley, Oral Gavage

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INTRODUCTION

The deleterious consequences of gestational alcohol exposure on skeletal elements are acknowledged in the medical literature [1,2]. Several studies suggest that low birth weight and impaired bone growth, as well as a decrease in mineralization in utero, may reduce bone mass while elevating the likelihood of fractures and osteoporosis with life progression [3-5].

A decrease in bone mass and disruption of trabecular and compact bone structure, causing a loss of mechanical strength and increased fracture risk are characteristic of osteoporosis [6]. The ability of the osseous tissue to withstand mechanical energy and fractures relies on both the quantity and quality of the osseous tissue. Bone quality is established by the structural and material properties which are influenced by the remodelling rate [7]. Bone remodelling is an ongoing process, comprising the renewal of the osseous tissue in which, osteoclasts resorb damaged or old bone, and osteoblasts deposit the new bone [8]. Alcohol is known to affect the balance of bone resorption and deposition, disrupting the internal micro-architecture, which results in diminished bone strength as seen in heavy alcohol consumption [9].

The strength of a bone and its structural properties are determined by shape and size as well as its microarchitecture [7]. The internal architecture of the osseous tissue is composed of 2 components trabecular and compact bone. Previous reports indicate that alcohol causes a reduction in compact and trabecular bone density and trabecular bone volume in young growing rats [10,11]. Similarly, colleagues found a reduced number of trabecular in the hamster femur following alcohol consumption [12]. This is thought to be due to the decline in osteoblast number and size as previously reported in alcohol-exposed rats [12]. A study by Ramadoss et al., 2006 found bone strength to be disturbed in gestational alcohol exposure [13]. As there are no comparable studies on postnatal bone strength in prenatal alcohol-exposed sheep, it remains unknown whether intrauterine alcohol disturbs the osseous tissue function in long-term postnatal life.

It is known that heavy alcohol consumption compromises bone quality both when consumed during gestation and when used in adult life [7,9,14]. Yet, it remains uncertain as to whether the deleterious effects on bone persist into adolescence and adult life in cases of intrauterine alcohol exposure during chronic heavy binge drinking. We questioned whether gestational alcohol exposure would affect bone strength and trabecular morphological parameters in 12-week-old rats when a binge drinking model of chronic alcohol is stopped at the initial 19 days of pregnancy. As such, we investigated this issue using bones of the arm and thigh (humerus and femur) in 12-week-old rats that were subjected to alcohol during gestation.

MATERIALS AND METHODS

Chemical regents and equipment

These chemicals were obtained as follows: ethanol absolute (1718733s, Sigma-Aldrich, Johannesburg SA) Paraformaldehyde (818715, Sigma-Aldrich, Johannesburg, SA). The equipment was obtained as follows microhaematocrit centrifuge (Haematokrit 210, Hetich, Germany), BioVision Ethanol Colorimetric Assay Kit (BioVision incorporation, Milpitas, USA), iMarkBio-radMicroplate Absorbance Reader (Bio-rad Laboratories Inc, USA), Nikon XTH 225/320 LC X-ray microtomography (Nikon, Tokyo Japan), Universal tensile testing machine (Z-X S 200V E R87 084,20 R87 084,20 SSM346-57320-44, Shimaduza, Welobie, SA).

Breeding and animal husbandry

The study received ethics approval from the Animal Ethics Screening Committee, University of Witwatersrand on 28 July 2015 (AESC 2015/27/15C). A total of ten female virgin Sprague Dawley (SD) rats weighing between 260-350g were used. All study animals were bred and kept at the Central Animal Services (CAS), University of Witwatersrand. These animals were maintained under pathogen free conditions, temperature-controlled environment (23°C ±2°C) and a 12-hour light/dark cycle. Pregnant dams were individually housed in plastic cages (L 430 mm x W 220 mm x H 200 mm), with free movement within the enclosures. The animals had unrestricted access to tap water and standard rodent diet.

Treatment with ethanol or saline and grouping of thepups

Time-mated (n=10) expecting Sprague Dawley dams were assigned to either the ethanol (n=5) or saline control (n=5) group. Gestation duration in a rat is 21-23 days therefore, the dams were treated with 0.015ml/g of 25.2% ethanol or 0.9% saline by oral gavage for the initial 19 days of pregnancy, respectively to avoid any problems with delivery of pupsas adapted from previous studies [2,11,14]. Since a relatively large dose was given once a day, this mimicked binge drinking. Two pups from each dam were used to obtain bilateral humerus (n=20) and femora (n=20). The body weight of the pups was recorded weekly to track the health of the pups weekly until termination. The 12-week-old rats were terminated with pentobarbital. To expose the humerus and femora, skin incisions were made on the respective limbs and muscles were meticulously teased away. The skeletal samples were then individually immersed and stored in 10% buffered formalin to continue fixation until further processing. Ten paired humerus and femora were obtained per group; however, one right humerus was excluded from the saline controls due to the formalin escaping from the storage container leaving the sample dry.

Determination of maternal blood alcohol levels

The tail vein was used to collect whole blood into heparinized microcapillary tubes for the initial 19 days of gestation, to determine the ethanol concentration in the blood of the dams, one hour after alcohol administration. Microcapillary tubes were spun in a microhaematocrit centrifuge (Haematokrit 210, Hetich, Germany) at 3000 rpm for 10 minutes. Plasma alcohol determination was carried out using the BioVision Ethanol Colorimetric Assay Kit (BioVision incorporation, Milpitas, USA) following the manufacturer's assay directives. All reactions and readings were done in an alcohol-free environment using an iMarkBio-radMicroplate Absorbance Reader (Bio-rad Laboratories Inc, USA).

Three-dimensional Micro-focus X-ray Computed Tomography (3D- µCT)

A Nikon XTH 225/320 LC X-ray microtomography was utilized for scanning the bilateral 12-week-old humerus and femora for 3D-µCT examination. To keep the samples steady during scanning, bones were mounted in low-density Styrofoam, placed in plastic tubes while allowing the X-rays to get to the sample with negligible absorption. The plastic container with the sample inside was positioned on a rotating manipulator in the scanning chamber for the scanning. The scanning parameters used are displayed in Table 1.

Table 1: Scanning parameters

Parameter	Value
X-ray voltage	70kv
X-ray current	400µA
Filter	1mm aluminium
Scanning resolution	20µm
Tomographic rotation	360degrees
Rotation step	1degree
Frame averaging	4
Scan duration	8 minutes

Parts of the humerus and femuras well as trabecular parameters studied

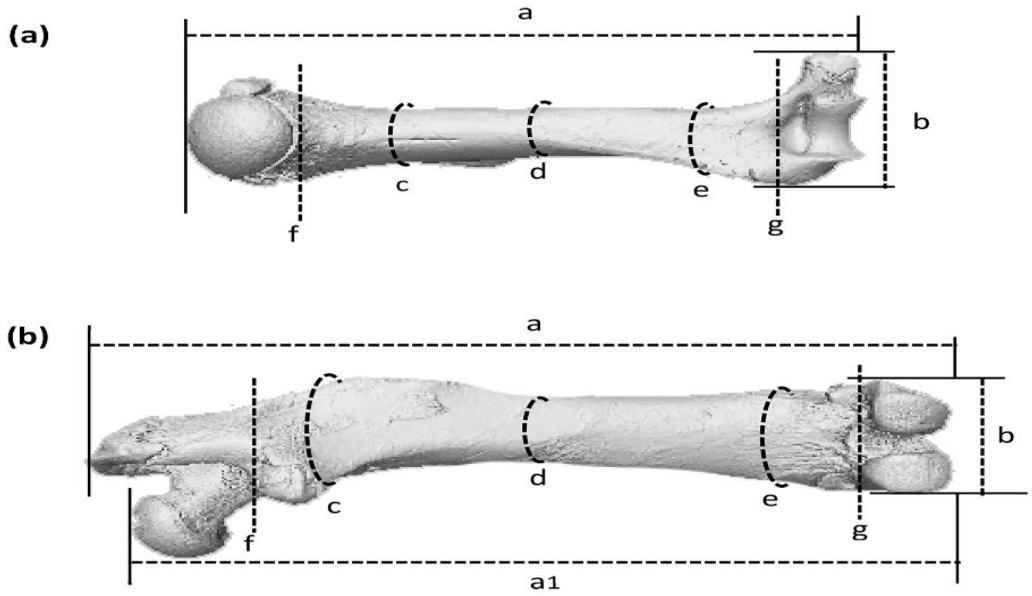
The VG studio software built in calliper was used to determine humeral and femoral osteometric parameters as in Table 2. From the humerus, the full bone length and epicondylar breath were taken, whereas full bone length, biomechanical and bicondylar length were measured from the femur (Figures1a & b.). The cross-sectional area, cortical area and medullary canal area of the shaft were measured at 3 positions: 25th (proximal), 50th (midshaft) and 75th (distal) percentile marks from both the humerus and femur (Figures1a & b).

Table 2: Osteometric parameter descriptions

Parameter	Description
Full humerus length	The maximum length that can be measured between the top of the humeral head and the most distant point on the distal humerus.
Full femur length	The maximum length measured between the top of the greater trochanter and the bottom of the farthest condyle
Biomechanical length	The maximum length from the top of the femoral head to the bottom of the farthest condyle.
Bicondylar breadth	the length between the medial most and lateral most points on the epicondyles

The trabeculae morphometry was investigated in the proximal and distal aspects in both bones. Following reconstruction, VG Studio Max®3.2 was used for data analysis as previously described [14]. The following trabecular parameters were assessed: bone volume to total volume (BV/TV), trabecular thickness (TbTh) trabecular number (TbN) and trabecular spacing (TbSp). Cross-sectional circumference, cross-sectional area and cortical area were determined.

Figure 1: Three-dimensional reconstruction of a 12-week-old humerus and femur showing the parts that were investigated, respectively. a, full humerus, and femur length, respectively; a1, biomechanical length of femur; b, bicondylar breadth of humerus; (c), (d), (e), 25th (proximal); 50th (midshaft) and 75th (distal) percentile marks, respectively; (f and g), proximal and distal region of interest for trabeculae morphological assessment of humerus and femur, respectively (Image courtesy of R. Ndou and D Pillay)



Three-point bending

Three-point bending test were conducted on the right femora (n =10 for both groups) and humerus (saline controls n = 9, ethanol group n = 10) of each rat. These bones were stored in 10% buffered formalin for 1 to 21 days before three-point bending tests. The load was applied at the midshaft, midway between two supports that were 15mm apart for the femur and 13 mm apart for the humerus. Before mechanical testing, various osteometric measurements were taken using a digital calliper. The femora and humerus were positioned so that bending occurred at the anteroposterior plane and mediolateral plane, respectively. Load-displacement curves were recorded at 5mm/min until failure.

Data analysis

The data were managed in Microsoft Excel 2016 (Microsoft Corporation) and all data were analysed using SPSS® version 27 (IBM®) except for Principal components analysis conducted using PAST (Paleontological Statistics, Paleontological Association, London, UK). The data was tested for normality using the Shapiro Wilks test. A 2-tailed independent samples t- test was performed as the data was parametric. Principal components analysis was conducted to assess how femur and humerus morphology parameters clustered. Significance level was set at $p < 0.05$. The statistical tests that were performed was on partially dependant measurements since only two pups from each dam were utilized.

RESULTS AND DISCUSSION

Blood alcohol concentration

The mean blood ethanol concentration was 170mg/dl in the ethanol group and negligible in the saline controls.

Weekly mass gained in 12-week-old rats

Mass at ages 3 and 12-weeks.

The initial mass was similar at 3 weeks of age for the ethanol (mean = 50.29g ±11.69) and saline controls (mean = 46.00g ±7.87) (t = -1,055, df = 22, p = 0,303). Similarly, no statistical mass differences were detected upon termination at 12 weeks of age with the saline controls (mean =345,79 ±92,76 being similar to the ethanol group (mean = 354,54 ±89,80) (t = -0,235, df =22, p =0,817).

Humerus

Bone length humerus and epicondylar breadth

The humerus length was significantly reduced in the ethanol group than the saline controls (t = 2.473, df = 41) (Figure 2a).

Also, the ethanol group exhibited a smaller epicondylar breadth than the saline control although not significant (Figure 2b) (t = 1.730, df = 41).

Humerus bone to total volume ratio (BV/TV)

In the proximal region, bone to total volume ratio (BV/TV) was lower in the ethanol group than the saline controls (t = 3.686, df = 42). Similarly, in the distal region, we found a significantly low bone to total volume (BV/TV) in the ethanol group than the saline controls (t = 4.340, df = 42) (Figure 2c).

Humerus trabecular thickness (TbTh)

In the proximal epiphysis, trabecular thickness was thinner in the ethanol group than the saline controls (t = -2.476, df = 42). (Figure 2d). Similarly, in the distal epiphysis, trabecular thickness was thinner in the ethanol group than the saline controls (t = -2.861, df = 42) (Figure 2d).

Humerus trabecular number (TbN)

Trabecular distribution stratified by study group membership had a similar pattern when arranged by the proximal and distal epiphyseal ends. The proximal region exhibited fewer trabeculae in the ethanol group than the saline control (t = 2.857, df = 42). This pattern of lower trabecular (TbN) in the ethanol group than the saline controls continued in the distal epiphysis (t = 3.059, df = 42) (Figure 2e).

Humerus trabecular spacing (Tbsp)

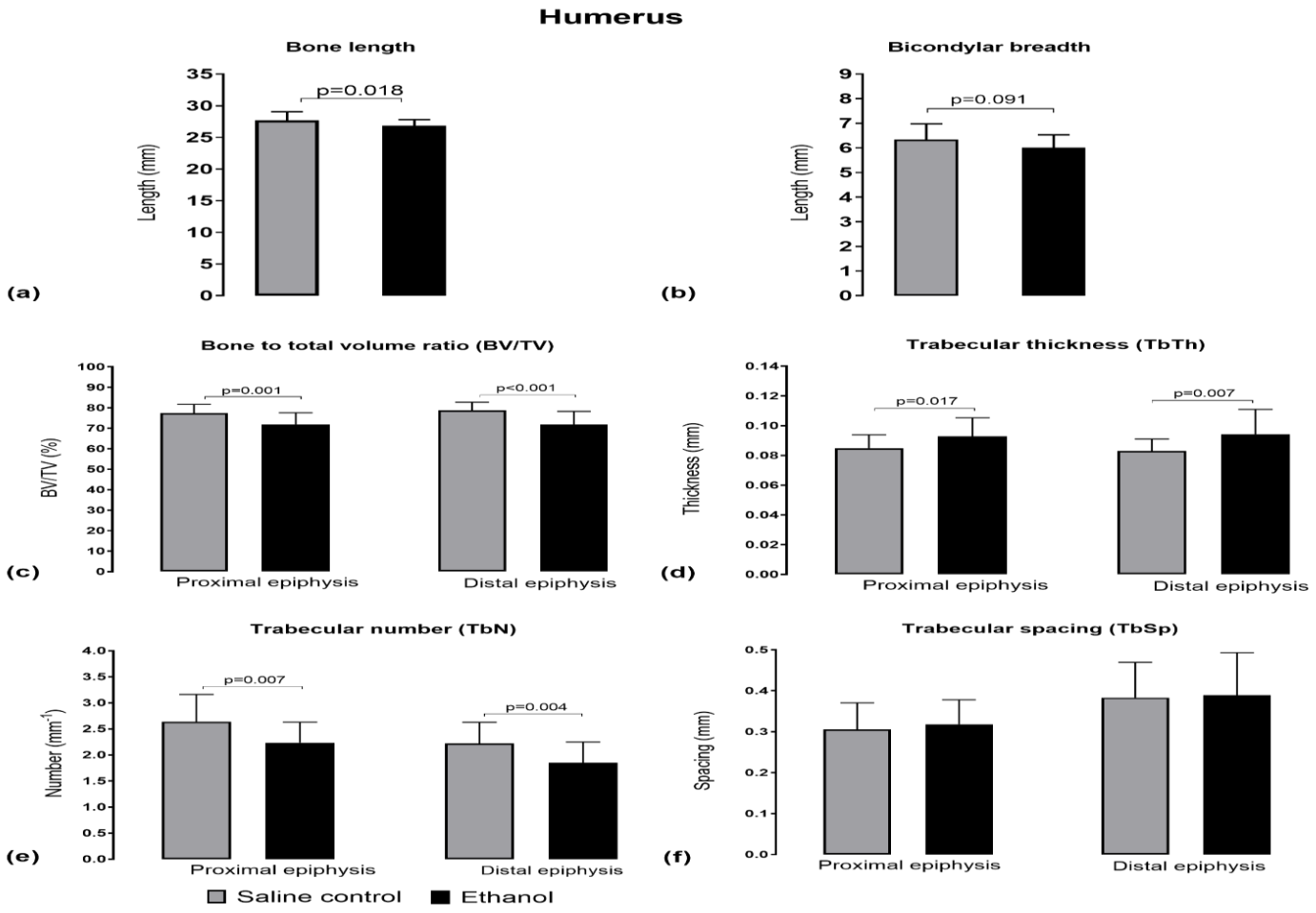
No group differences in trabecular spacing (TbSp) occurred in the proximal and distal extremities of the humerus (Figures 2f and 3a and b). In the proximal region, trabecular spacing (TbSp) was marginally lower in the saline compared to the ethanol group (t = -0.669, df = 42, p = 0.507). Again, in the distal region, the trabecular spacing was similar between the two groups (Figures 2f and 3c and d).

Humerus cross-sectional area, cortical area, and medullary canal area

Proximal (25th percentile mark)

The proximal cross-sectional area in the ethanol group was like that of the saline controls (t = -0.743, df = 46, t = 0.46, p = 0.461) (Figure 4a). Similarly, the medullary canal area in the ethanol and saline groups was not significantly different (t = -1.509, df = 46, p = 0.138) (Figure 4a). With respect to the cortical area in this region, a similar pattern of no significant difference between the groups was observed, with the ethanol group exhibiting similarities to the saline controls (t = 0.584, df = 46, p = 0.562).

Figure 2: Osteometric measurements and trabeculae morphometric parameters of the humerus**.** (a), full bone length; (b), epicondylar breadth. Represented as means for the saline control (grey bar) and ethanol group (black bar) (c), bone to total volume (BV/TV); (d), trabeculae thickness (TbTh); (e), trabeculae number (TbN) and (f), trabeculae spacing (TbSp) represented at the proximal and distal epiphysis of the humerus. Error bars represent standard deviation



Midshaft (50th percentile mark)

The midshaft cross-sectional area was similar in the ethanol group and saline controls (t = 1.591, df = 46, p = 0.118) (Figure 4b). In contrast, the medullary canal area was smaller in the ethanol group than the saline group (t = 2.431, df = 46, p = 0.018). Regarding the cortical area in this region, the ethanol group (mean = 4.04mm2 ±0.79) exhibited a similar value compared to the saline (t = 0.392, df = 46, p = 0.697) (Figure 4b). *Distal (75th percentile mark).*

Figure 3: Trabecular morphology in the humerus. Representative slices of (a), proximal end in a saline control showing more trabeculae and less spacing; (b), proximal end of the ethanol group showing fewer trabeculae and wider spacing); (c), distal end of saline control showing more trabeculae and less spacing; (d), distal end of the ethanol group showing fewer trabeculae and wider spacing. Scale bar represents 1mm and applies to all.

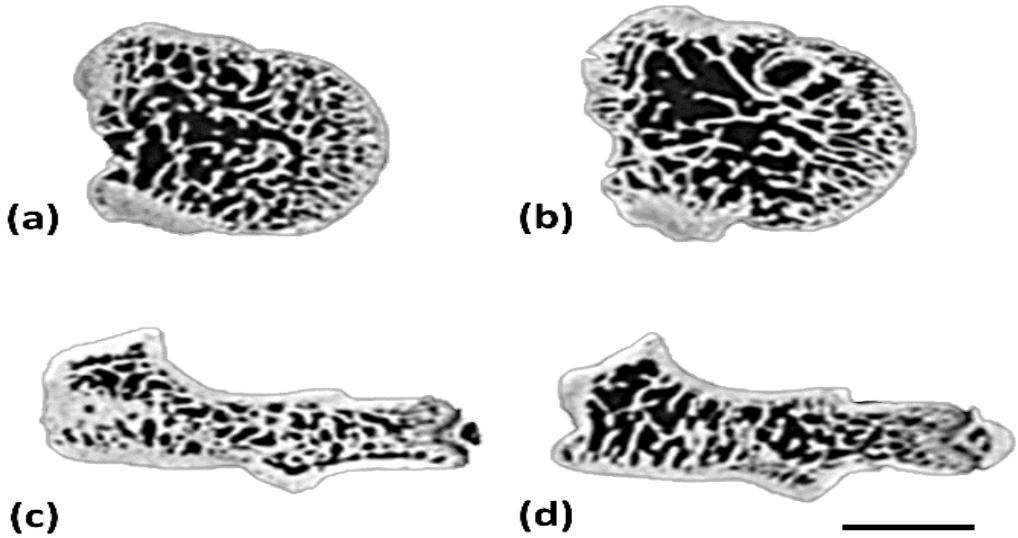
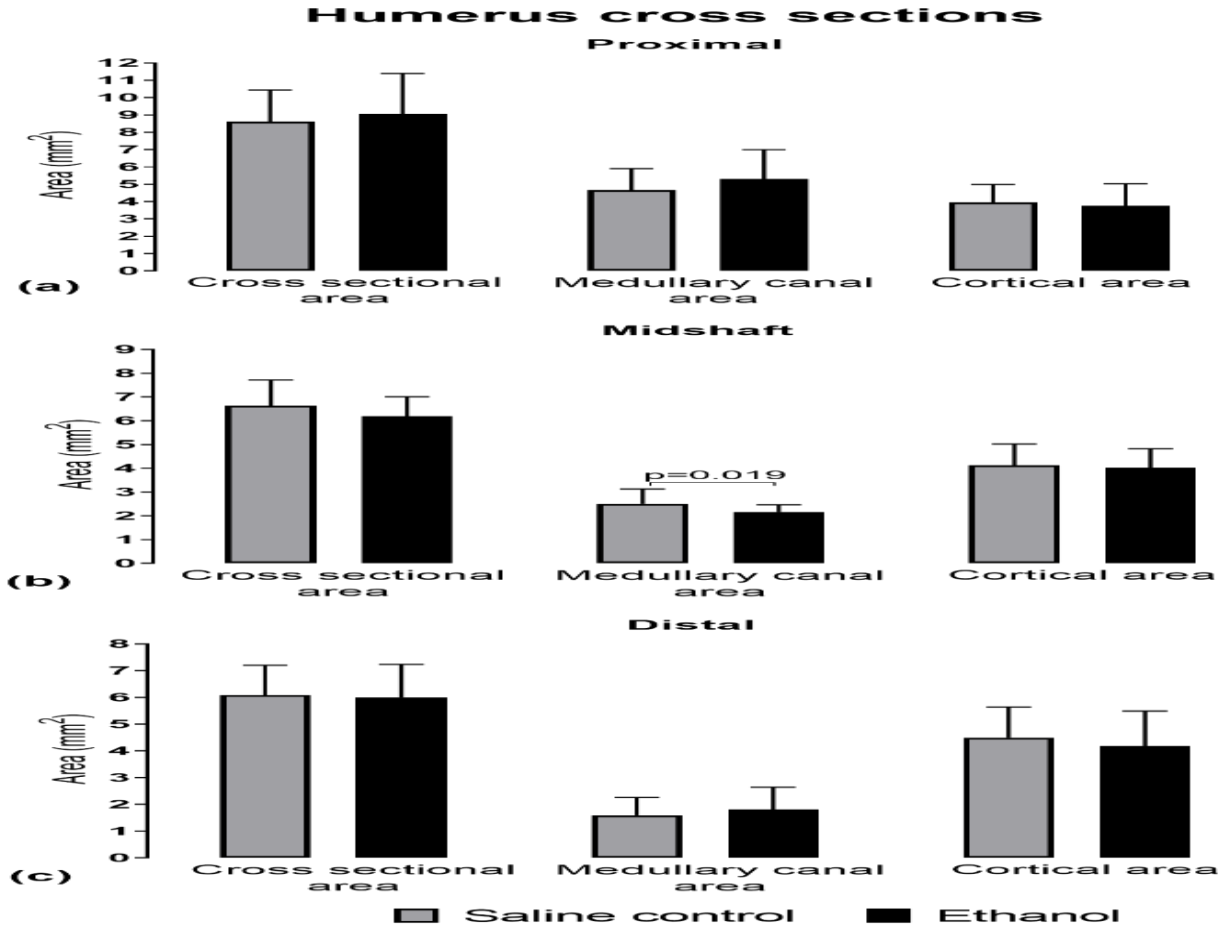


Figure 4: Cross-sectional area and medullary canal areas and cortical areasof the humerus. Represented as means for the saline controls (grey bar) and ethanol group (black bar); (a), at the 25th percentile mark; (b), 50th percentile mark; (c), 75th percentile mark. Error bars represent standard deviation.



The distal cross-sectional area was similar in the ethanol group (mean = 6.00mm2 ±1.24) compared to the saline controls (mean = 6.08mm2 ±1.12) (t = 0.251, df = 46, p =0.803). (Fig. 4c). The distal medullary canal area was also similar in the ethanol group (mean = 1.82mm2 ±0.83) in comparison to the saline controls (mean = 1.59mm2 ±0.63) (t = -1.042, df = 46, p = 0.303) (Fig. 4c). With respect to the cortical area in this region, the ethanol group (mean = 4.18mm2 ±1.31) exhibited this pattern of being like the saline groups (mean = 4.49mm2 ±1.14) (t = 0.879, df = 46, p = 0.384) (Figure 4c).

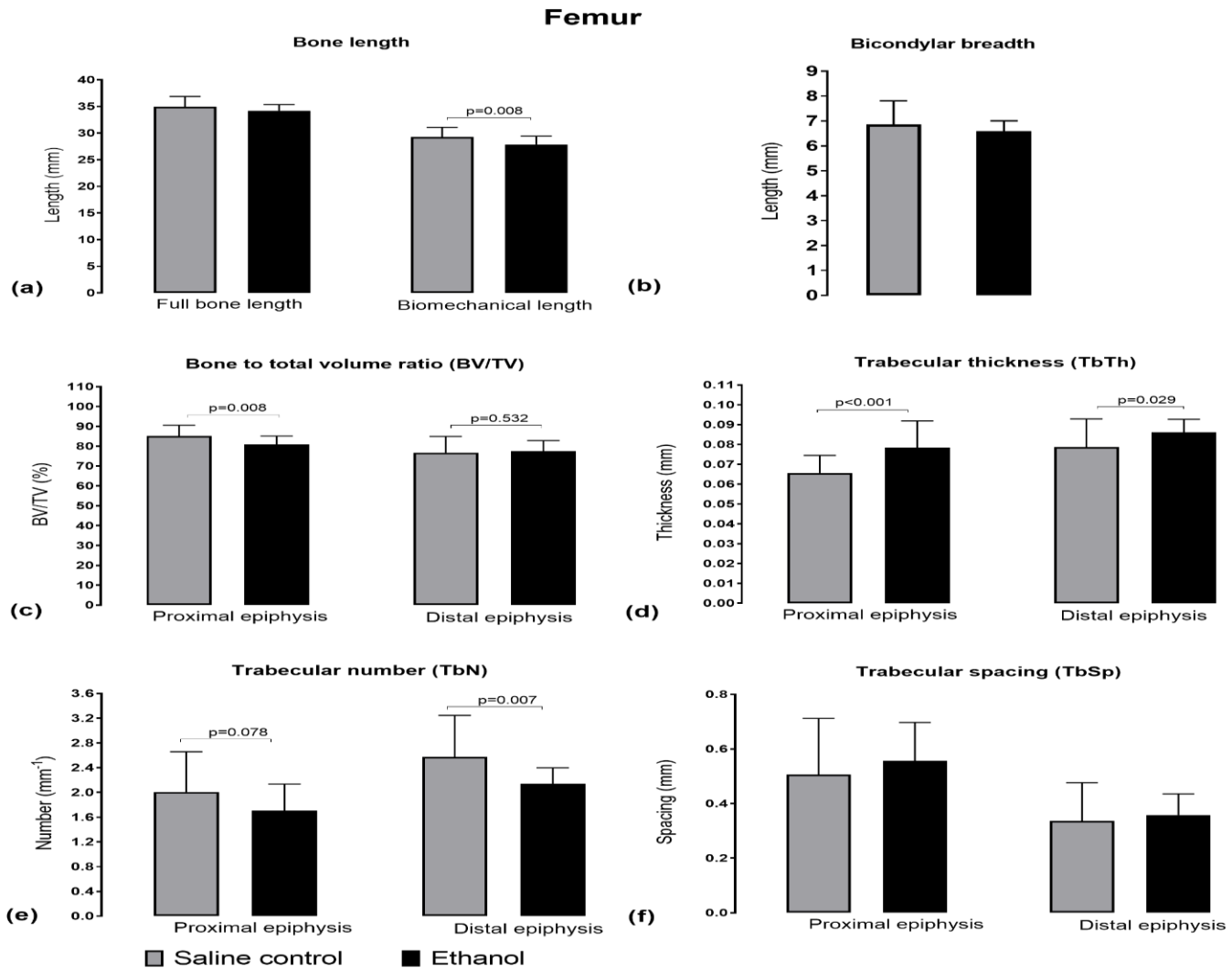
Humerus tensile strength using 3-point bending tests

We detected no group differences in the bone weight between the saline controls and the ethanol group (t = -0.461, df =17, p = 0.651). The bones head diameter in the ethanol group and the saline controls were similar (t = 0.026, df = 17, p = 0.980). The maximum force and break force were again similar between the ethanol group and saline controls (t = -0.276, df = 17, p= 0.786 and t = -0.295, df = 17, p = 0.771, respectively). With respect to the amount of time required to fracture the bones when applying force (maximum time), the 2 groups exhibited similarities (t = 0.643, df = 17, p = 0.529) (Table 3). The energy to fracture was similar between the groups (t = -0.618, df = 17, p =0.544).

Table 3: Osteometric measurements and tensile strength of the humerus

Name	Control	N	Mean	SD
Bone weight (g)	Saline control	9	0.489	0.078
	Ethanol	10	0.532	0.269
Head diameter (mm)	Saline control	9	4.717	0.373
	Ethanol	10	4.713	0.239
Maximum force (N)	Saline control	9	66.29	17.26
	Ethanol	10	68.35	15.26
Maximum displacement (mm)	Saline control	9	0.788	0.184
	Ethanol	10	0.826	0.141
Maximum time (Sec)	Saline control	9	10.71	3.543
	Ethanol	10	9.908	1.696
Break force (N)	Saline control	9	65.80	17.75
	Ethanol	10	68.03	15.19
Energy to fracture	Saline control	9	25.74	8.964
	Ethanol	10	28.07	7.479

Figure 5: Osteometric and trabeculae morphometric parameters of the femur. (a), full bone length; (b), biomechanical length; (c), bicondylar breadth. Represented as means for the saline control (grey bar) and ethanol group (black bar) (c), bone to total volume (BV/TV); (d), trabeculae thickness (TbTh); (e), trabeculae number (TbN) and (f), trabeculae spacing (TbSp) represented at the proximal and distal epiphysis of the humerus. Error bars represent standard deviation.



Femur

Femur bone length

The mean full bone length of the femur was similar among the two groups in the study (t = 1.545, df = 43, p = 0.130) (Figure 5a). However, group differences in the biomechanical length occurred, being shorter in the than the saline controls (t = 2.822, df = 43) (Figure 5a).

Femur bicondylar breadth

The bicondylar breadth showed marginal differences between the two groups in the study (Figure 5b). The ethanol group had smaller but not significant bicondylar breadth than the saline controls (t = 1.243, df = 43, p = 0.221).

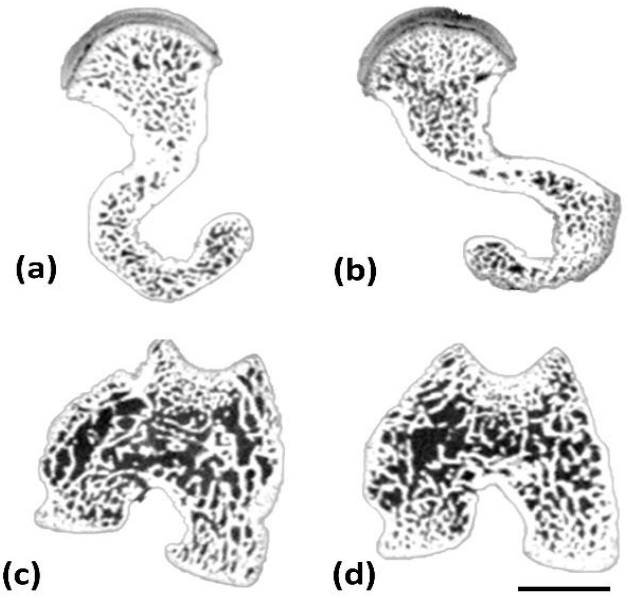
Femur bone to total volume ratio (BV/TV)

In the proximal epiphysis, group differences in the bone to total volume ratio (BV/TV) were significantly smaller in the ethanol group than the saline controls (t = 2.786, df = 43) (Figure 5c). Similarly, the distal epiphysis displayed a marginally lower bone to total volume ratio (BV/TV) in the ethanol group than the saline controls with no significant differences detected (t = -0.630, df = 43) (Figure 5c).

Femur trabecular thickness (TbTh)

The mean trabecular thickness (TbTh) was thinner in the ethanol group than saline controls in both epiphyseal ends. In the proximal epiphysis, it was significantly greater in the ethanol group than the saline controls (t = -3.793, df = 43) (Figure 5d). Similarly, the distal epiphysis, had significantly greater trabecular thickness (TbSp) for the ethanol group compared to saline (t = -2.252, df = 43, p = 0.029) (Figure 5d).

Figure 6: Trabecular morphology in the femur. (a), proximal end of saline control showing more trabeculae and less spacing; (b), proximal end of the ethanol group showing fewer trabeculae and wider spacing; (c), distal end of saline control showing more trabeculae and less spacing; (d), distal end of the ethanol group showing fewer trabeculae and wider spacing. Scale bar represents 2mm and applies to all.



Femur trabecular number (TbN)

Trabecular distribution assessed by study group showed a varied pattern when stratified by the proximal and distal epiphyseal ends of the femur. In the proximal region the trabecular number (TbN) was not statistically different for the ethanol compared to the saline group (Figure 5e) (t = 1.808, df = 43). Conversely, the distal epiphysis, exhibited fewer trabeculae (TbN) in the ethanol group than the saline (t = 2.842, df = 43) (Figure 5e).

Femur trabecular spacing (TbSp)

The trabecular spacing (TbSp) was similar in the study groups for both the proximal and distal extremities of the femur. In the proximal region no differences were detected between the ethanol group and the saline controls (t = -0.953, df = 43, p = 0.346) (Figures 5f and 6a and b). In a similar pattern, the distal region the trabecular spacing did not show major differences in the ethanol group and saline controls (t = -0.640, df = 43, p = 0.525) (Fig. 5f and 6c and d).

Femur cross-sectional area, cortical area and medullary canal area

Proximal (25th percentile mark)

The proximal cross-sectional area was smaller in the ethanol group than the saline controls (t = 2.897, df = 46). We also observed a smaller medullary canal area in the ethanol group compared to the saline controls (t = 4.735, df = 46). Concerning the cortical area in this region, the ethanol group exhibited a similar pattern to the saline controls (t = 0.508, df = 46, p = 0.614) (Figure 7a).

Midshaft (50th percentile mark)

The midshaft cross-sectional area varied in the two groups studied with the ethanol group showing a significantly lower than the saline controls (t = 2.118, df = 46) (Fig. 7b). The medullary canal area in the ethanol group although not significant showed a smaller medullary canal area than the saline group (t = 1.440, df = 46, p = 0.157) (Fig. 7b). Regarding cortical area in this region, the ethanol group exhibited similarities to the saline (mean = 6.59mm2 ±1.05) (Fig. 7b) (t = 1.023, df = 46, p = 0.312).

Distal (75th percentile mark)

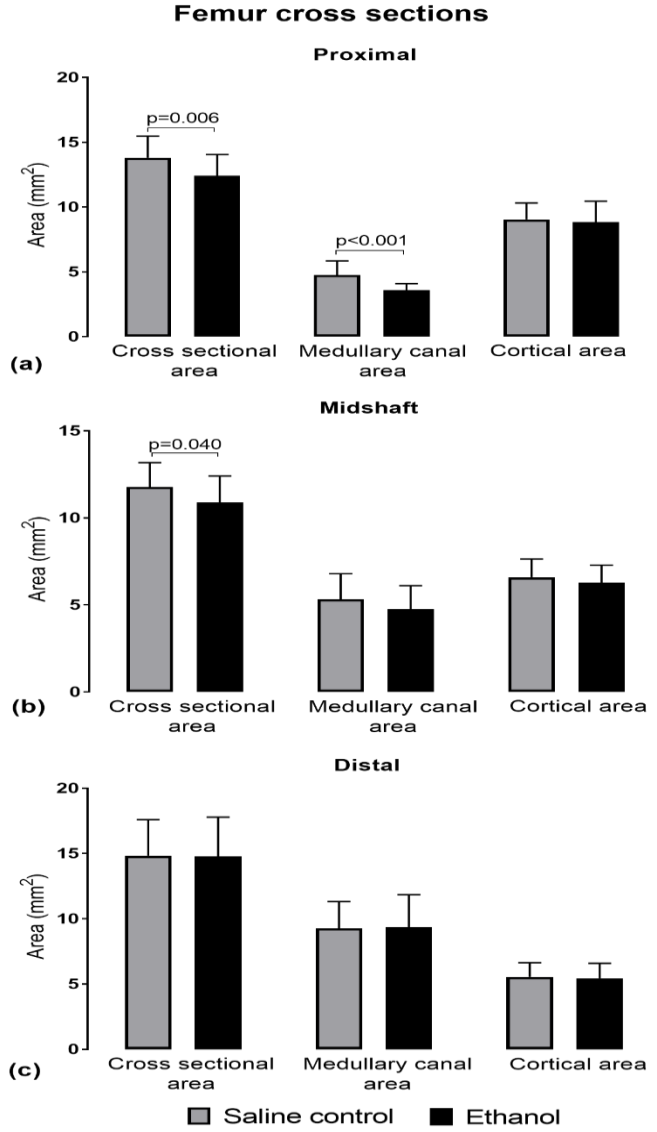
The distal cross-sectional area was similar in the ethanol group than the saline controls (t = 0.044, df = 46, p = 0.965) (Figure 7c). Also, the medullary canal area was similar between the groups (t = -0.130, df = 46, p = 0.897) (Figure. 7c). This observation of similarity between the ethanol group and saline controls continued for the cortical area in this bone region (mean = 5.43mm2 ±1.15; and 5.55mm2 ±1.08, respectively) (t = 0.377, df = 46, p = 0.708).

Tensile strength of the femur using 3-point bending tests.

We observed a similar value and no significant bone weight in the ethanol group than the saline control as indicated in Table 4 (t = 0.280, df = 18, p = 0.783). Also, the maximum force and break force were similar in the ethanol group and the saline controls (t =

0.028, df = 18, p =0.978 and t = 0.440, df = 18, p = 0.665, respectively). With respect to the maximum time required to fracture, the ethanol group displayed a lower value than saline controls (Table 4). Again, there were no statistical group differences detected (t = 0.346, df = 18, p = 0.733). The energy to fracture was similar in the ethanol group in comparison to the saline control (t = 0.384, df = 18, p = 0.706)

Figure 7: Cross-sectional area and medullary canal areas and cortical areas of the femur. Represented as means for the saline controls (grey bar) and ethanol group (black bar); (a), at the 25th percentile mark; (b), 50th percentile mark; (c), 75th percentile mark. Error bars represent standard deviation.



Major parameters for the ethanol and saline controls

Principal components analysis was conducted to assess how femur and humerus morphology parameters clustered. These variables were the bone length, BV/TV, TbTh, TbN, TbSp. Two components were used based on the eigenvalues above 1 criterion. Following rotation, the first component contributed 91.4%, whereas only 8.6% of the variance was from the second component. In component 1, Femur BV/TV and TbTh, Femur biomechanical length, Humerus BV/TV and TbTh, Femur Distal TbN, Humerus length, HumerusTbN were the main contributing factors (Table 5 and Figure 8).

Table 4: Osteometric measurements and tensile strength of the femur.

		N	Mean	SD
Bone weight (g)	Saline control	10	1.034	0.2
	Ethanol	10	1.011	0.155
Maximum Force (N)	Saline control	10	86.33	10.89
	Ethanol	10	86.19	11.61
Maximum Displacement (mm)	Saline control	10	1.598	0.459
	Ethanol	10	1.519	0.555
Maximum Time (Sec)	Saline control	10	19.16	5.506
	Ethanol	10	18.22	6.662
Break Force (N)	Saline control	10	85.75	10.86
	Ethanol	10	83.67	10.26
Energy to fracture	Saline control	10	68.06	20.62
	Ethanol	10	63.93	27.03

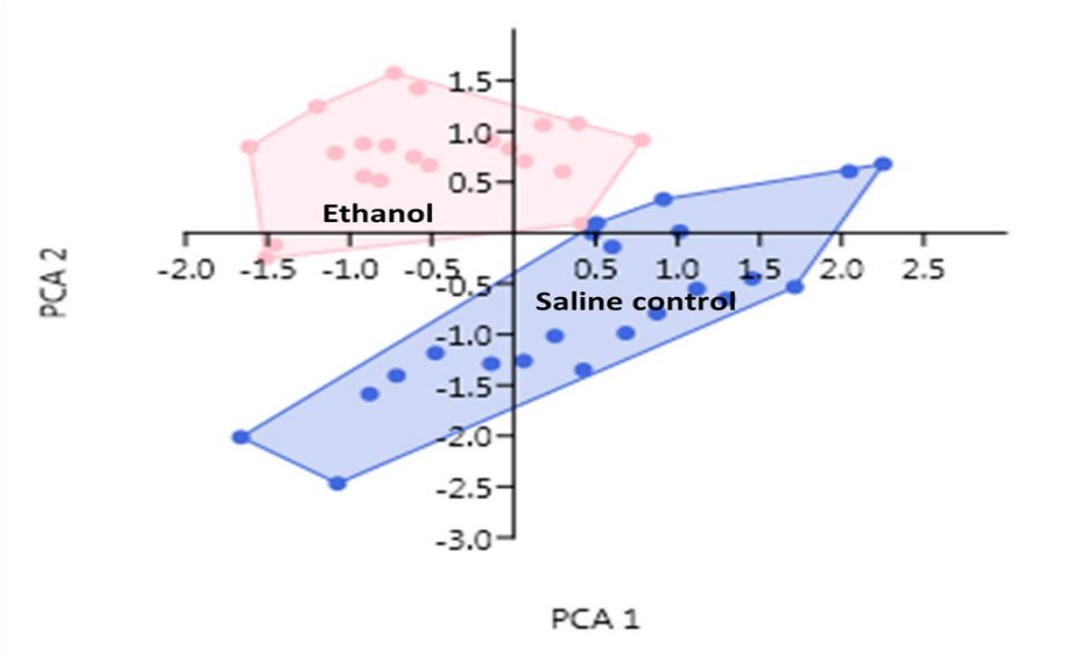
Table 5: Varimax rotation of the principal components analysis of femur and humerus**.** The contributing variables are arranged in descending order according to component 1, with major contributors in bold (above ±0.3). BV/TV = bone to total volume ratio; TbN = Trabecular number; TbTh = Trabecular thickness; TbSp = Trabecular spacing.

Parameter	PC 1(91.4%)	PC 2 (8.6%)
Femur distal BV/TV	**0.971**	0.238
Femur biomechanical length	**0.445**	0.060
Femur proximal BV/TV	**0.435**	0.150
Humerus proximal BV/TV	**0.423**	0.383
Humerus proximal TbN	**0.405**	-0.137
Femur distal TbN	**0.370**	-0.018
Humerus length	**0.350**	0.087
Humerus distal TbN	**0.326**	0.303
Humerus epicondylar breadth	0.299	-0.026
Femur bicondylar breadth	0.230	-0.109
Femur length	0.216	0.051
Femur proximal TbN	0.108	0.232
Humerus distal TbSp	0.101	-0.369
Femur proximal TbSp	-0.014	-0.149
Humerus proximal TbSp	-0.049	0.036
Femur distal TbSp	-0.058	0.011
Femur distal TbTh	**-0.318**	-0.017
Humerus proximal TbTh	**-0.394**	-0.047
Humerus distal TbTh	**-0.418**	-0.075
Femur proximal TbTh	**-0.572**	-0.067
Humerus distal BV/TV	**-0.933**	0.359

The theory that gestational alcohol exposure has postnatal detrimental effects on bone was tested with a binge drinking rat model. These effects include shorter and osteoporotic bones that easily fracture [15,11,16]. The present study found a shorter humerus, whereas only the biomechanical length was shortened in the femur. In both bones, cortical and medullary dimensions remained unaffected except for the smaller midshaft in the ethanol group, coupled with smaller trabecular morphometric parameters.

The shorter humerus found in this study corroborate previous reports [2]. However, only the femoral biomechanical length was shorter in the ethanol group. Biomechanical length represents the load bearing parts of the femur, supporting the idea that gestational ethanol exposure shortens bones, potentially translating to short stature observed in FAS children [17].

Figure 8: Principal components analysis. The extent of disparity between the ethanol group and saline control for the femur and humerus with respect to optometric measurements and trabecular morphometry is illustrated.



Both bones showed fewer and thinner trabeculae in the ethanol group, accompanied by a smaller bone to volume ratio. Therefore, prenatal alcohol exposure affected trabecular parameters in the humerus and femur alike. These 2 bones are not widely studied as most studies on prenatal alcohol exposure focus on the tibia, radius, and ulna [18 - 20].

A smaller medullary canal area was observed in the midshaft of both the bones. This could have compensated for possible bone weakness as the cortical thickness was proportionally larger with a reduction in the medullary canal area. Since the midshaft is also the part that was tested for strength in 3-point bending tests, this could explain the absence of bone strength group differences. This finding of a small medullary canal area obtained in the study fails to support the theory that gestational alcohol causes weaker bones. However, it supports the idea of potential catch up growth in children born small for age following prenatal alcohol exposure.

Studies suggest that offspring of mothers who drink in pregnancy may have a recovery period in postnatal life with nutritional interventions [21 - 23]. It is possible that the rat chow consumed by all animals in this study may have mitigated the detrimental effects of intrauterine alcohol ingestion, resulting in the observed minor group differences postnatally.

PCA revealed that BV/TV, TbN and TbSp were the most affected parameters in both bones. This agrees with our hypothesis that prenatal alcohol exposure would cause fewer, thinner trabecular and lower bone to total volume ratio. Only in the humerus was length part of the principal components, showing that the effects of alcohol in fetal stages persisted to postnatal week 12. This supports the idea that postnatal recovery from gestational alcohol effects is not guaranteed.

This study is the first to investigate bone strength in bones exposed to ethanol prenatally. No bone strength differences were found using 3-point bending tests. This agrees with the observation that cortical area and medullary canal area did not contribute to the principal components for the ethanol and saline controls. However, the findings do not support the observation that FAS children are prone to fracture. This may be consequential to the daily single dosage of alcohol given unlike having the alcohol in drinking water ensuring multiple alcohol intake daily.

CONCLUSION

Alcohol resulted in shorter bones in the study. Both the humerus and the femur exhibited that gestational alcohol exposure alters trabecular morphometric parameters as total bone volume and trabecular number were smaller with thinner trabeculae. This suggests that the internal bone structure may not recover postnatally after gestational alcohol exposure. A similar finding was made on both the humerus and femur, suggesting that either the humerus or the femur is a suitable candidate for similar studies. However, the tensile strength was similar in the ethanol and saline groups, supporting the proposition of postnatal recovery when adequate nutrition is provided

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