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Growth rate of bones in rat foetal alcohol syndrome

Innocent Chima Nwaogu*

Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Nigeria Nsukka, Enugu State, Nigeria

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ABSTRACT

The effect of maternal alcohol consumption during pregnancy on the growth rate of humerus and femur were studied in 168 offspring of rats. Experimental foetal alcohol syndrome was produced by giving 10% ethanol (v/v) in water to eight-week-old female albino rats for 2 weeks, then 20% ethanol (v/v) for a further 3 weeks, and mating them overnight. When confirmed pregnant, the alcohol concentration was increased to 30% until delivery, when alcohol consumption was stopped. The control group was not given alcohol and both groups were fed *ad libitum*. Birth masses, as well as lengths of humerus and femur, of the control rats were significantly higher ($P<0.01$) than those of pre-natally alcohol-exposed rats at 3, 5, 7, 9, 11 and 14 weeks of age. There was no significant difference ($P>0.05$) between litter sizes of both groups. The relative growth rates of femur were significantly higher ($P<0.01$) in the experimental group than in the control group. These results suggest that the low birth masses observed in pre-natally alcohol-exposed rats did not depend on litter size. They also demonstrated that alcohol consumption by pregnant rats adversely affected the growth rates of humerus and femur of their offspring pre-natally, and this effect persisted throughout the period of this study.

Key words: albino rat, alcohol, bones, foetus, growth rate

Introduction

Alcohol-related problems now rank among the world's major public health concerns, not only in most industrialised countries but also in developing countries (MICHELLE, 1980). Alcohol is among some of the potentially

* Contact address:

Dr. I. C. Nwaogu, Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria, E-mail: misunn@aol.com

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harmful factors that are transmitted directly to the embryo (NODEN and DELAHUNTA, 1985). This was confirmed by reports of ROMERT and MATHIESSEN (1992) that samples of blood collected from ethanol-treated rats and their foetuses approximately 3 hours after intake showed equal concentration of ethanol in both, with an average value of 50 mg/100 ml. It has been reported that alcohol consumed by mothers during pregnancy, particularly during the first trimester, causes many congenital defects in offspring of both human and experimental animal models (HANSON et al., 1976; ABEL, 1982a, 1982b; SULIK and JOHNSTON, 1985; IHEMELANDU, 1984; CLARREN et al., 1985).

In experimental Foetal Alcohol Syndrome (FAS) the allometric growth of skeletal muscles and various viscera was adversely affected in rats (NWAOGU and IHEMELANDU, 1999a; 1999b). LEE and LEICHTER (1980) reported that retardation of growth during pre- and post-natal life was not due to under-nutrition of the mother. These authors further concluded that their results, coupled with the fostering studies of ABEL and DINTCHEFF (1978) and that of ABEL and GREIZERSTEIN (1979) on foetal growth, supported their hypothesis that maternal alcohol consumption adversely affected the regulatory mechanism for growth during embryonic or foetal development and that the effect persists after birth.

Since the first cases of FAS were identified (JONES et al., 1973) the long-term effect of FAS, or the most recently described foetal effect, is yet to be determined (STREISSGUTH et al., 1985). In the studies cited above the extent to which maternal alcohol consumption affects the growth of bones in offspring was not quantified. This work was therefore designed to study the growth of humerus and femur of pre-natal alcohol exposed rats in comparison with a control group.

Materials and methods

The method employed in producing FAS in this study was similar to that of LEE and LEICHTER (1980). Six-week-old female albino rats obtained from the Faculty of Veterinary Medicine, University of Nigeria breeding facility were housed in metal cages with 4 animals in each. They were provided with commercial rat feed and water *ad libitum*. They were

acclimatized in our laboratory for two weeks before the commencement of the study. Fourteen female animals were allocated to each of the experimental and control groups. The control group received ordinary water throughout the period of the study.

At the beginning of the ninth week of age, rats in the experimental group were given 10% ethanol (v/v) in drinking water, which was raised to 20% ethanol in the eleventh week, and continued for 3 more weeks.

At thirteen weeks of age the animals were mated overnight and the vaginae examined next morning for plugs. Day 1 of pregnancy was regarded as the day vaginal plugs were found or sperms observed in the vaginal washing next morning. In pregnant dams of the experimental group the alcohol content of the drinking water was adjusted to 30% ethanol (v/v). Pregnant animals were placed one in a cage until delivery. At birth the litter size for each animal in both groups was adjusted to six by fostering the offspring from mothers with large litter sizes to those with small litter sizes within the same group.

Immediately after delivery the alcohol was replaced with ordinary water for the experimental group. A total of 168 offspring (84 males and 84 females) were used for the study. At 3, 5, 7, 9, 11 and 14 weeks of age 7 males and 7 females were randomly selected from each group and killed by cervical dislocation at the atlanto-occipital joint.

Quantitative measurement. At birth the masses of individual offspring were determined and the average calculated for each dam. This was taken as the birth mass for that particular litter; group mean birth mass was calculated by adding the litter birth masses of the 14 dams and dividing by 14. At death the humeri and femora were dissected out and immersed in water overnight for easy separation of soft tissues. The length of the bones from the articular head to trochlear was then measured. The length of left and right limb bones were determined and their mean taken as the bone length. The relative growth rates of the bones were determined by dividing their 5-, 7-, 9-, 11- and 14-week length with their respective length at 3 weeks of age.

Mean and standard errors were calculated for each group. Student ‘t’ test was used to determine the significance of observed differences.

Results

When comparison was made using Students 't' test there was no significant difference (P>0.05) between the litter sizes of both control and alcohol-exposed rats. On the other hand, the birth masses of control pups were significantly higher (P<0.01) than those of alcohol-exposed pups (Table 1.)

Table 1. Comparison of litter sizes and birth masses (g) using Students 't' test

Parameters	Control Rats	Prenatal alcohol exposed rats	Probability of significance (t value)
Litter Size	6.21 ± 0.42	6.43 ± 0.29	0.42
Birth mass (g)	6.00 ± 0.25	5.11 ± 0.64	4.43**

Values represent mean ± SE for each measurement. Degrees of freedom = 26 for all parameters. **P<0.01

Analysis of lengths of humeri showed that those of the control rats were significantly greater than those of alcohol-exposed rats at 3, 5, 7, 9, 11 and 14 (P<0.01) weeks of age. Both groups were similar at 9 (P>0.05) weeks of age (Table 2.)

Table 2. Comparison of lengths of humerus (cm) using Students 't' test

Age (weeks)	Control rats	Alcohol exposed rats	Probability of significance (t value)
3	1.53 ± 0.02	1.40 ± 0.02	5.29**
5	1.88 ± 0.01	1.75 ± 0.04	3.20**
7	1.97 ± 0.02	1.86 ± 0.03	3.38**
9	2.15 ± 0.02	2.09 ± 0.03	1.94
11	2.26 ± 0.02	2.14 ± 0.03	3.65**
14	2.32 ± 0.02	2.16 ± 0.01	5.96**

Values represent Mean ± SE for each measurement. Degrees of freedom = 26 for all parameters. **P<0.01

Comparison of femur lengths indicated that those of control offspring were significantly greater than those of alcohol-exposed offspring at 3, 5, 7, 9, 11 and 14 (P<0.01) weeks of age (Table 3.)

Table 1. Specification of myomorphus mammals examined by renoculture and microscopic agglutination acording to the trapping area with corresponding results

Table 3. Comparison of lengths of femur (cm) using Students 't' test

Age (Weeks)	Control rats	Alcohol exposes rats	Probability of significance (t value)
3	1.77 ± 0.02	1.54 ± 0.03	6.88**
5	2.35 ± 0.02	2.14 ± 0.03	5.18**
7	2.51 ± 0.03	2.37 ± 0.04	2.62**
9	2.77 ± 0.02	2.68 ± 0.03	2.20*
11	2.92 ± 0.03	2.75 ± 0.03	4.17**
14	3.08 ± 0.03	2.79 ± 0.03	7.42**

Values represent Mean ± SE for each measurement. Degrees of freedom = 26 for all parameters. **P<0.01 *P<0.05

Comparison of the relative growth rates of humerus showed that there was no significant difference (P>0.05) between both groups, except at 9 weeks of age when those of the experimental group were significantly higher than (P<0.01) those of controls. On the other hand, the femora of alcohol exposed rats showed significantly higher relative growth rates than those of the controls at 5, 7, 9, 11 and 14 (P<0.01) weeks of age (Table 4.)

Table 4. Comparison of relative growth rates of bones using Students 't' test

Humerus			
Age (weeks)	Control rats	Alcohol exposed rats	Probability of significant (t values)
5	1.23 ± 0.01	1.25 ± 0.02	0.72
7	1.29 ± 0.01	1.33 ± 0.01	1.62
9	1.41 ± 0.01	1.49± 0.02	4.10**
11	1.48 ± 0.01	1.53 ± 0.02	2.00
14	1.52 ± 0.02	1.54 ± 0.01	1.44
Femur			
5	1.33 ± 0.01	1.39 ± 0.02	2.52**
7	1.42 ± 0.02	1.54 ± 0.02	4.24**
9	1.56± 0.01	1.74 ± 0.02	7.61**
11	1.65 ± 0.02	1.79 ± 0.02	5.40**
14	1.74 ± 0.02	1.82 ± 0.02	3.35**

Values represent Mean ± SE for each measurement. Degrees of freedom = 26 for all parameters. **P<0.01

Discussion

The results of this study have demonstrated that alcohol consumed by rats before and during pregnancy retarded the growth of humerus and femur of their offspring and this effect persisted for a long time in their post-natal life. This was evident when the length of their bones as well as their relative growth rates were used as indices of growth. Comparison between the lengths of humerus and femur of both groups showed that those of control rats were greater than those of alcohol-exposed rats throughout the period of study. This finding is similar to reports that the clinical features of human FAS during neonatal life include short stature, low mass, small head circumference, psychomotor retardation, proximal weakness and muscle hypotrophy (MARTIN et al., 1976; SULIK and JOHNSTON, 1985). The observation further supports the report that plasma insulin-like growth factors (IGF-1) in ethanol-treated offspring were reduced by 14 to 40% when compared to control rats at birth, 10 and 20 days of age (BRESSE et al., 1993), since IGF-1 is induced by growth-hormone and its plasma concentration parallels the postnatal growth rate (GANNONG, 1987).

The relative growth rates of femur of alcohol-exposed offspring were greater than those of the controls, while those of humerus were similar in both groups. Normally, the relative growth rates of both bones are expected to behave alike. The reason why the humerus should behave differently from the femur is not obvious. NWAOGU and IHEMELANDU (1999a) reported the same relationship between growth coefficients of biceps brachii, (forelimb muscle) and quadricips femoris (hind limb muscle) in pre-natally alcohol-exposed rats when compared with the controls.

This is probably as a result of different functions performed by forelimb and hind limbs in support of body mass of animal in a standing position (IHEMELANDU and IBEBUNJO, 1992). Bones have centres of proliferation which continue to grow post-natally. The post-natal linear growth of bones is by epiphyseal growth plate. This process continues until puberty because sex hormones inhibit linear growth by favouring growth plate closure through accelerating metaphyseal osseous replacement and inhibiting proliferative chondrocytes (BANKS, 1993). Puberty age is eight weeks in rats (BENNET and VICKERY, 1970) but rats continue to grow, although at a declining rate,

throughout life (GANNONG, 1987). This may be the reason for continuous increase in length of the bones observed in both groups throughout the period of this study.

The higher relative growth rates exhibited by the bones of alcohol-exposed offspring post-natally may be an attempt to compensate for growth retardation which they suffered pre-natally. In spite of this, their absolute lengths were still shorter than those of controls. This is similar to reports that pre-natal alcohol exposure adversely affects the skeletal neuromuscular junctions (OKAMATO and WALESKI, 1992), histomorphology of the liver (ROMERT and MATHIESSEN, 1983a; 1983b; 1984; 1987; 1992) and allometric growth of skeletal muscles and viscera (NWAOGU and IHEMELANDU, 1999a; 1999b).

The control rats weighed significantly more than the alcohol-exposed rats at birth and there were no significant differences between the litter sizes of both groups. This suggests that the higher birth masses observed in the control group did not arise from any difference between the litter sizes of both groups. This further supports the observations of LEE and LEICHTER (1980) that reducing the number of pre-natal alcohol-exposed offspring per dam does not enhance their physical growth or maturation.

In conclusion, this study has demonstrated that the low birth masses observed in offspring of alcohol-exposed rats were not dependent upon their litter size. Alcohol consumption by pregnant rats adversely affected the growth rates of humerus and femur of their offspring pre-natally, and this effect persisted until puberty (at eight weeks) and throughout the period of this study.

References

ABEL, E. L. (1982a): Fetal alcohol syndrome II: Human studies, Boca Raton, PL, CRC Press, Inc.

ABEL, E. L. (1982b): Fetal alcohol syndrome III: Animal studies. Boca Raton, PL, CRC Press, Inc.

ABEL, E. L., B. A. DINTCHEFF (1978): Effects of prenatal alcohol exposure on growth and development in rats. J. Pharmacol. Exp. Therapeutics 207, 916-921.

ABEL, E. L., H. B. GREIZERSTEIN (1979): Ethanol-induced prenatal growth deficiency changes in fetal body composition. J. Pharmacol. Exp. Therapeutics 211, 666-671.

BANKS, W. J. (1993): Osteogenesis. In: Applied veterinary histology (3rd ed.) Mosby Year Book Inc. Missouri.

BENNET, J. P., B. H. VICKERY (1970): Puberty and development of gametes in mice and rats. In: Reproduction and breeding techniques for laboratory animals. Lea and Febinger, Philadelphia.

BRESSE, C. R., A. D'COSTA, R. L. INGRAM, J. LENHAM, W. E. SONNATAG (1993): Long-term suppression of insulin-like growth factor-1 in rats after in uteroethanol exposure; relationship to somatic growth. J. Pharmacol. Exp. Therapeutics 264, 448-456.

CLARREN, S. K., D. M. BOWDEN, S. ASTELY (1985): The brain in the fetal alcohol syndrome. Alcohol Hlth. Res. Wrld 10, 20-30.

GANNONG, W. F. (1987): Physiology of growth. In: Review of Medical Physiology (13th ed.) Prentice-Hall International Inc. Connecticut.

HANSON, J. W., K. L. JONES, D. W. SMITH (1976): Fetal alcohol syndrome. Experience with 41 patients. J. Am. Med. Assoc. 235, 1458-1460.

IHEMELANDU, E. C. (1984): Effect of maternal alcohol consumption on pre-and postnatal muscle development of mice. Growth 48, 35-43.

IHEMELANDU, E. C., C. IBEBUNJO (1992): Comparison of effect of castration on the development of postural and non-postural muscles of mice. Acta anatomica 143, 283-288.

JONES, K. L., D. W. SMITH, C. N. ULLELAND, A. P. STREISSGUTH (1973): Pattern of malformations in offspring of chronic alcohol mothers. Lancet 1, 1267-1271.

LEE, M., J. LEICHTER (1980): Effect of litter size on the physical growth and maturation of offspring of rats given alcohol during gestation. Growth 44, 327-33.

MARTIN, J. T., R. CLARA, C. H. CEUTERICK, C. I. JORRIS (1976): Is congenital fibre type disproportion a true myopathy? Acta Neurologica Belgium 76, 335-334.

MICHELLE, H. (1980): The International Development Centre Reports 9 (1), 15.

NWAOGU, I. C., E. C. IHEMELANDU (1999a): Effects of maternal Alcohol consumption on the allometric growth of muscles in foetal and neonatal rats. Cells tissues Organs 164, 167-173.

NWAOGU, I. C., E. C. IHEMELANDU (1999b): Allometric growth of visceral organs in foetal alcohol sydrome of rats. Nigerian Vet. J. 20, 30-39.

NODEN, D. M., A. DeLAHUNTA (1985): Causes of congenital malformations. In: The Embryology of Domestic Animals. Development al Mechanisms and Malformations. Williams and Wilkins. Baltimore.

OKAMATO, M., J. L. WALESKI (1992): Chronic ethanol effects on developing neonatal skeletal neuromuscular junctions *in vivo*. In: Alcohol and Neurobiology. Brain development and Hormone regulation. (Watson R. R., Ed.), Academic press.

ROMERT, P., M. E. MATHIESSEN (1983a): Alcohol-induced injury of mitochondria in hepatocytes of mini-pig fetuses. Virchows Archives Abt. Zellphathologia 399, 299-305.

ROMERT, P., M. E. MATHIESSEN (1983b): Ethanol-induced changes of granular endoplasmic reticulum in hepatocytes of mini-pig foetuses. Acta Pathol. Microbiol. Scand. 91.

ROMERT, P., M. E. MATHIESSEN (1984): Fine structure of hepathocytes from mini-pig and mini-pig foetuses exposed to ethanol *in vivo*. Acta Anatomica 120, 190-195.

ROMERT, P., M. E. MATHIESSEN (1987): Quantitative study of hepatocytes of mini-pigs and mini-pig foetuses exposed to ethanol *in vivo*. Acta Anatomica 130, 227-241.

ROMERT, P., M. E. MATHIESSEN (1992): Ultrastructuraland morphometric study of hepatocytes from near-term mini-pig foetuses exposed to ethanol in vivo. *Acta Anatomica* 143, 301-305.

STREISSGUTH, A. P., S. K. CLARREN, K. L. JONES (1985): A natural history of foetal alcohol syndrome: a 10-year follow-up of eleven patients. Lancet 2, 85-92.

SULIK, K. K., M. C. JOHNSTON (1985): Sequence of developmental alterations following acute ethanol exposure in mice: craniofacial features of the fetal alcohol syndrome. Am. J. Anatomy 166, 257-269.

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SAŽETAK

Istra•en je učinak alkohola na stupanj rasta humerusa i femura u 168 mladunčadi podrijetlom od štakorica kojima je alkohol davan za vrijeme gravidnosti. Pokusni fetalni alkoholni sindrom bio je izazvan davanjem 10% etanola (v/v) u vodi albino štakoricama starosti 8 tjedana, a zatim 20% etanola (v/v) u tijeku sljedeća 3 tjedna. Štakorice su potom bile parene preko noći. Nakon potvrđene gravidnosti, koncentracija alkohola bila im je povećana na 30% sve do koćenja, kada se prestalo s davanjem alkohola. Kontrolna skupina nije dobivala alkohol. Obje skupine bile su hranjene *ad libitum*. Nakon partusa u mladunčadi je praćena tjelesna masa te dužina humerusa i femura. U kontrolnih štakora okotna masa te dužina humerusa i femura bile su značajno veće (P<0,01) u odnosu na štakore podrijetlom od tretiranih ženki i to 3., 5., 7., 9., 11. i 14. tjedna starosti. Nije utvrđena značajna razlika u broju mladunčadi između dvije skupine.(P>0,05). Relativna brzina rasta femura bila je značajno veća (P<0,01) u pokusne u usporedbi s kontrolnom skupinom. Rezultati ukazuju na činjenicu da mala tjelesna masa mladunčadi prenatalno izložene alkoholu nije ovisila o veličini legla. Dokazano je da je alkohol u gravidnih ženki nepovoljno utjecao na stupanj rasta humerusa i femura u prenatalnom razdoblju njihove mladunčadi, te da se taj učinak nastavio i nakon okota sve do kraja pokusa.

Ključne riječi: albino štakor, alkoholizam, kosti, fetus, rast