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COMPARATIVE STUDY ON THE GROWTH PATTERNS OF INTERNAL ORGANS OF MALE AND FEMALE MICE AT THREE STAGES OF DEVELOPMENT

IGWEBUIKE*[1], U. M. and ASOGWA, M. N.

[1]Department of Veterinary Anatomy
University of Nigeria Nsukka Nigeria

*Correspondence

SUMMARY

This study investigated the growth of various organs in 36 male and 36 female mice using increase in weight as the index of growth. The mice were randomly selected from the offspring of the same breeding stock. They were given a commercially prepared diet and drinking water *ad libitum* until they were sacrificed at 3, 6 and 12 weeks of age. At each age, 12 males and 12 females were sacrificed by decapitation at the atlanto-occipital joint. The live body weight of each mouse was determined using a mettler top-loader weighing machine. Following death, skeletal muscles (triceps brachii and gastrocnemius muscles) and some internal organs (lungs, heart, kidneys, liver and spleen) were dissected and their weights were determined using a mettler beam balance. Humerus and femur were also dissected from each mouse and their lengths were determined using a venier caliper. The muscle mass index (milligram muscle weight per gram body weight) was calculated for each of these muscles. The Relative Organ Weight (ROW), expressed as percentage of body weight contributed by each internal organ was calculated. The muscle mass indices of the muscles of male and female mice were not significantly different ($p>0.05$) at 3 weeks of age. However, at 6 and 12 weeks of age, there were significant differences in the muscle mass indices of biceps brachii ($p<0.01$) and gastrocnemius ($p<0.05$) muscles of male and female mice. There were no significant differences ($p>0.05$) between the relative weights of organs of male and female mice at 3 weeks of age. The relative weights of the heart ($p<0.05$), liver ($p<0.01$), spleen ($p<0.05$) and kidneys ($p<0.01$) were significantly greater in the males at 6 weeks of age. At 12 weeks of age, the relative weights of the heart ($p<0.05$) and the kidneys ($p<0.01$) remained significantly greater in the male mice, but the relative weights of the lungs ($p<0.05$) and the spleen ($p<0.01$) were significantly greater in females. The study therefore, demonstrated that although sex differences were not evident in the relative weights of these organs at 3 weeks of age, there were sex differences in the body weights and growth patterns of muscles, bones and internal organs of male and female mice at 6 weeks and 12 weeks of age. It was suggested that these sex differences might have arisen from the possible significant influence of sex hormones on the growth and development of these organs in both male and female mice.

KEY WORDS: Mice, organs, growth, sex differences

INTRODUCTION

Several factors are thought to influence the process of growth at different stages in the course of development. Balinsky (1970) suggested that the process of growth may be controlled by the genotype in two different ways. Firstly, the genetic constitution may act directly on the ability of cells to grow and proliferate. Alternatively, the gene action may be indirect through its primary effect to modify the differentiation of the anterior lobe of the hypophysis responsible for the secretion of growth hormones. This accounts for the hereditary differences in the sizes of animals. In addition to growth hormone, the anterior lobe of the hypophysis is also responsible for the release of gonadotrophic hormones. The

stimulatory action of these hormones on the gonads results in the production of sex hormones namely, testosterone and oestrogen in males and females respectively (Warren *et al,* 1975; Armstrong and Papkoff, 1976).

It has been suggested that hormones particularly, androgens may have a direct or at least, an indirect stimulatory influence on the development of muscles (Goldspink, 1972). Thus, the muscles of adult male mice were found to be larger than those of adult female mice (Goldspink and Rowe, 1968). Cheek *et al* (1968) made a similar observation in rats. The sex difference was found to be due to greater increase in the sizes of myofibres in males than females. There were no differences in the number of myofibres in the muscles of both sexes of mice (Rowe and Goldspink, 1969; Aberle and Doolittle, 1976). Studies by Venable (1966a), as well as, Ihemelandu and Ibebunjo (1992) revealed that retardation of muscle growth resulted from the castration of male rats and mice. Venable (1966a; 1966b) demonstrated that replacement therapy using testosterone, following castration of males, caused considerable hypertrophy of the fibres in rat *levator ani* muscles. It was noted that the hypertrophy of the muscles was a result of an increase in myofibrilar material of the fibres. There was no change in the number of fibres either during the replacement therapy or during the atrophy of the muscles following castration. At the cellular level, Buresova *et al.* (1969) reported an increased incorporation of labeled amino acids into muscle proteins following administration of androgens to both intact and castrated animals. Androgens have therefore been shown to increase the rate of protein synthesis in most muscles and so, influence the rate and extent of development of muscle fibres.

Large doses of oestrogen administered within the first 5 days of postnatal life have been found to produce a depressant effect on the development of various muscles in sexually immature female rabbits (Ihemelandu, 1984). Ihemelandu (1988) observed lack of differences in the muscle weights of pre-pubertal male and female mice at 4 weeks of age. The same author reported greater muscle weights in the males than the females at 5 and 6 weeks of age. According to him, this period coincided with the period of significant increase in the secretion of testosterone and oestrogen in the males and females respectively; hence, the differences in their muscle weights. Furthermore, in the presence of oestrogen, there were no differences between the muscle mass of developing male and female mice (Ihemelandu, 1981). These observations suggest that the smaller muscle mass of adult females when compared to adult males may be attributed to the inhibitory effect of oestrogen on muscle development in the females. It has been demonstrated that oestrogen brought about this inhibition of muscle mass development by limiting the size of individual muscle fibres (Ihemelandu, 1984).

Although sex-differences and the influence of sex hormones on muscle growth and development have been extensively studied as indicated by the review above, there is paucity of information on the possible sex-differences in the pattern of growth and development of internal organs. The present study was therefore designed to investigate the probable influence of gender on the growth of internal organs and bones of

mice. It seeks to study the growth patterns of these organs in male and female mice at pre-pubertal, pubertal and adult age groups, using increase in weight as the index of growth (Balinsky, 1970).

MATERIALS AND METHODS

Five male and 15 female mice used as the breeding stock in this study were randomly selected from a group of inbred albino mice maintained for research in the Department of Veterinary Anatomy, University of Nigeria Nsukka. They were fed a commercially prepared diet and drinking water *ad libitum*. Following acclimatization period of 2 weeks, the 15 female mice of the breeding stock were mated with the 5 males at a ratio of one male to three females. After Parturition, the neonates were left with their dams until they were weaned at 21 days of age. 72 mice (36 males and 36 females) were randomly selected from the offspring of the breeding stock and were used as the experimental animals in this study. They were housed in cages with aluminum bottom and screened top according to sex. Each cage housed 6 mice. These mice were fed the commercially prepared diet and drinking water *ad libitum* until they were sacrificed at 3, 6 and 12 weeks of age. These ages were chosen to represent pre-pubertal, pubertal and adult age groups respectively (Bennet and Vickery, 1970). At each age, 12 males and 12 females were sacrificed by decapitation at the atlanto- occipital joint.

Prior to sacrificing each mouse, the live body weight was determined using a mettler top-loader weighing machine. Following death, triceps brachii of the two forelimbs and gastrocnemius muscles of the two hindlimbs were dissected and weighed. The mean weight of the right and left muscles was determined. Internal organs such as the heart, lungs, liver, spleen and kidneys were dissected from each mouse and the weight of each organ was determined using a mettler beam balance. The muscle mass index (milligram muscle weight per gram body weight) was determined for each muscle. Similarly, the relative organ weight expressed as the percentage of body weight contributed by each internal organ (gram organ weight per gram body weight multiplied by 100) was determined. These allometric parameters yielded size-independent dimensional constants and thus, allowed comparison of organ weights over a wide range of body sizes (Stahl, 1965). Two bones namely, humerus and femur were dissected from both fore and hind limbs of each mouse. The bones were immersed in water overnight to facilitate separation of soft tissues from them. The length of each femur from the articular head to the trochlear, and humeral epicondyles were measured using a venier caliper. The mean length ± S. E of left and right bones was determined.

Statistical analysis

Means and standard errors were calculated for each group of observations. The data obtained were subjected to statistical analysis using Student's 't' Test to determine whether significant differences exist between observed means (Fisher and Yates, 1967).

RESULTS

Comparison of the body weights of male and female mice (Table I) indicated that although there was no significant difference ($p>0.05$) between the sexes at 3 weeks of age, the male mice were significantly

heavier ($p<0.01$) than the females at 6 and 12 weeks of age. Furthermore, the weights of muscles, as well as, the length of bones, which did not differ significantly ($p>0.05$) between male and female mice at 3 weeks of age, differed significantly ($p<0.01$) between sexes at 6 and 12 weeks of age. The males possessed heavier muscles and longer bones than the females. However, the length of femur was similar ($p>0.05$) in male and female mice at 12 weeks of age. Comparison of the muscle mass indices of the muscles of male and female mice (Table II) showed that there were no significant differences ($p>0.05$) between sexes at 3 weeks of age.

TABLE I: Comparison of body weights (g), muscle weights (mg) and length of bones (cm) using Student's 't' Test

Age (weeks)	(N)	Parameters	Males	Females	t-value
3 weeks	(12)	Body weight (g)	9.3 ± 0.04	9.2 ± 0.4	0.167
		Triceps brachii muscle (mg)	26.9 ± 1.3	27.9 ± 1.7	0.447
		Gastrocnemius muscle (mg)	39.4 ± 2.9	38.2 ± 2.8	0.069
		Humerus (cm)	0.44 ± 0.01	0.45± 0.01	0.524
		Femur (cm)	0.53 ± 0.01	0.56± 0.02	1.049
6 weeks	(12)	Body weight (g)	27.3 ± 0.8	18.3 ± 0.4	9.368**
		Triceps brachii muscle (mg)	83.8 ± 3.0	50.6 ± 1.6	9.477**
		Gastrocnemius muscle (mg)	144.2 ± 4.6	88.3 ± 3.7	9.036**
		Humerus (cm)	0.76 ± 0.02	0.61 ± 0.01	7.035**
		Femur (cm)	0.99 ± 0.02	0.83 ± 0.01	8.390**
12 weeks	(12)	Body weight (g)	34.0 ± 1.2	27.3 ± 1.0	4.202**
		Triceps brachii muscle (mg)	134.2 ± 4.8	84.2 ± 3.5	8.068**
		Gastrocnemius muscle (mg)	199.1 ± 9.1	149.7 ± 6.4	4.249**
		Humerus (cm)	0.89 ± 0.01	0.82 ± 0.02	3.283**
		Femur (cm)	1.19 ± 0.02	1.16 ± 0.02	1.049

Values represent mean ± standard error for each measurement.
df = 22 **p<0.01

TABLE II: Comparison of muscle mass indices (mg/g) of muscles using Student's 't' Test

Age	(N)	Muscles	Males (mg/g)	Females (mg/g)	t-value
3 weeks	(12)	Triceps brachii	2.89 ± 0.07	3.02 ± 0.09	1.083
		Gastrocnemius	4.20± 0.17	4.11 ± 0.15	0.421
6 weeks	(12)	Triceps brachii	3.07 ± 0.07	2.78 ± 0.08	2.508**
		Gastrocnemius	5.28± 0.07	4.84 ± 0.19	2.093*
12 weeks	(12)	Triceps brachii	3.95 ± 0.08	3.08 ± 0.06	8.699**
		Gastrocnemius	5.85± 0.16	5.48 ± 0.11	1.809*

Values represent mean ± standard error for each measurement.
df = 22 *p<0.05 **p<0.01

The muscles mass indices of triceps brachii ($p<0.01$) and gastrocnemius ($p<0.05$) muscles were significantly greater in the male mice than the females at 6 and 12 weeks of age. The weights of the heart ($p<0.01$) and the spleen ($p<0.05$) were significantly different between male and female mice at 3 weeks of age, but the lungs, liver and kidneys were not significantly different ($p>0.05$) in the two sexes of mice at this age (Table III). The weights of heart ($p<0.01$), lungs ($p< 0.05$), liver ($p<0.01$), spleen ($p<0.01$) and kidneys ($p<0.01$) were significantly greater in male mice than females at 6 weeks of age (Table III). At an adult age of 12 weeks, the weights of heart ($p<0.01$), liver ($p<0.01$) and kidneys ($p<0.01$) were still significantly greater in the males. There were no significant differences ($p>0.05$) in the weights of spleen and lungs of male and female mice at this age (Table III). Comparison of the relative organ weights of the organs of male and female mice (Table IV) revealed that no significant differences ($p>0.05$) existed between sexes at 3 weeks of age. The only exception was the relative weight of the heart, which was significantly greater ($p<0.05$) in male than female mice. The relative weight of the heart ($p<0.05$), liver ($p<0.01$), spleen ($p<0.05$) and kidneys ($p<0.01$) were significantly greater in the males at 6 weeks of age (Table IV). However, at this same age, the relative weight of the lungs did not differ significantly ($p>0.05$) between sexes. The relative weight of the heart ($p<0.05$) and kidneys ($p<0.01$) remained significantly greater in the males at 12 weeks of age, while that of the lungs ($p<0.05$) and spleen ($p<0.01$) were significantly greater in the females at the same age. There was no significant difference ($p>0.05$) in the relative weight of the liver in both sexes of mice at 12 weeks of age (Table IV).

TABLE III: Comparison of weights of internal organs (g) using Student's 't' Test

Age	(N)	Visceral Organs	Males (g)	Females (g)	t-value
3 weeks	(12)	Heart	0.07 ± 0.003	0.06 ± 0.002	2.909**
		Lungs	0.12 ± 0.01	0.12 ± 0.01	0
		Liver	0.45 ± 0.03	0.44 ± 0.03	0.247
		Spleen	0.05 ± 0.003	0.04± 0.004	1.915*
		Kidneys	0.13 ± 0.005	0.13± 0.01	0
6 weeks	(12)	Heart	0.15 ± 0.01	0.09 ± 0.003	9.277**
		Lungs	0.25 ± 0.02	0.18 ± 0.01	2.357*
		Liver	1.66 ± 0.05	0.89 ± 0.04	12.314**
		Spleen	0.16 ± 0.01	0.08 ± 0.01	6.850**
		Kidneys	0.40 ± 0.01	0.20± 0.01	15.216**
12 weeks	(12)	Heart	0.18 ± 0.01	0.13 ± 0.004	5.270**
		Lungs	0.27 ± 0.02	0.03 ± 0.04	0.671
		Liver	1.56 ± 0.08	1.24 ± 0.05	3.185**
		Spleen	0.12 ± 0.01	0.12 ± 0.01	0
		Kidneys	0.54 ± 0.02	0.32 ± 0.02	7.690**

Values represent mean ± standard error for each measurement.
df = 22 *p<0.05 **p<0.01

TABLE IV: Comparison of the relative organ weights of internal organs (%) using Student's 't' Test

Age	(N)	Visceral Organs	Males (%)	Females (%)	t-value
3 weeks	(12)	Heart	0.71 ± 0.03	0.64 ± 0.02	1.780*
		Lungs	1.26 ± 0.08	1.31 ± 0.12	0. 332
		Liver	4.80 ± 0.11	4.76 ± 0.11	0.246
		Spleen	0.49 ± 0.03	0.47± 0.04	0.383
		Kidneys	1.36 ± 0.03	1.41± 0.03	1.172
6 weeks	(12)	Heart	0.54 ± 0.02	0.50 ± 0.02	1.713*
		Lungs	0.91 ± 0.08	0.99 ± 0.06	0.766
		Liver	6.07 ± 0.10	4.87± 0.13	7.125**
		Spleen	0.57 ± 0.03	0.43 ± 0.05	2.451*
		Kidneys	1.47 ± 0.05	1.11± 0.02	5.896**
12 weeks	(12)	Heart	0.51 ± 0.01	0.48 ± 0.01	2.171*
		Lungs	0.80 ± 0.04	1.07 ± 0.13	1.949*
		Liver	4.55 ± 0.12	4.55 ± 0.06	0.0
		Spleen	0.35 ± 0.02	0.44± 0.02	3.567**
		Kidneys	1.60 ± 0.04	1.16 ± 0.03	8.569**

Values represent mean ± standard error for each measurement.
df = 22 *p<0.05 **p<0.01

DISCUSSION

The results of this study demonstrated that although the body weights of mice did not differ significantly between males and females at 3 weeks of age, sex differences were apparent at 6 weeks and 12 weeks of age, with the male mice weighing more than the female mice. Meara (1947) had reported that in most species, there exist sex differences in body weights of animals, the males appearing to be slightly heavier than females. Similarly, Ihemelandu (1981) observed greater body weights in adult male mice when compared to adult female mice.

It was observed in this study that both the absolute weights, as well as, the muscle mass indices of the muscles of male mice were similar to those of female mice at the pre-pubertal age of 3 weeks. However, at 6 weeks and 12 weeks of age, these parameters were consistently greater in male mice than female mice. This agrees with reports by previous workers (Goldspink and Rowe, 1968; Rowe and Goldspink, 1969; Aberle and Doolittle, 1976; Ihemelandu 1981), that the muscles of adult male mice are invariably larger than those of adult female mice. This disparity between the muscle mass of male and female mice had been attributed to the stimulatory influence of testosterone on muscle growth in the males (Venable, 1966a; 1966b; Goldspink, 1972), and the inhibitory role of oestrogen on muscle growth in the females (Ihemelandu, 1980; 1981). Similar to the report by Ihemelandu (1988), it was observed in this study that sex differences did not exist in the weights of muscles of male and female mice until puberty. The sex difference observed from 6 weeks of age may therefore have resulted from the effect of sex hormones on the muscles. It has been reported that this age coincided with the period of significant increase in the secretion of testosterone and

oestrogen in male and female mice respectively (Ihemelandu, 1988).

The observations of the present study indicated that the length of long bones did not differ significantly between male and female mice at 3 weeks of age, but at 6 weeks and 12 weeks of age, the males possessed longer bones than the females. Riesenfeld (1976) suggested the probable influence of sex hormones on bone growth. He reported that the administration of an overdose of oestrogen to male rats produced a depressant effect on the growth of tibia. Furthermore, Fried and Smith (1962), as well as, Wettenhall and Roche (1965) used large doses of oestrogen to retard longitudinal growth in girls prior to full sexual maturity. Generally, oestrogen is known to inhibit linear growth by favoring growth plate closure, inhibiting proliferation of chondrocytes and accelerating metaphyseal osseous replacement.

The results of this study demonstrated that at 3 weeks of age, the weights of the liver, kidneys, lungs and the spleen were not significantly different ($p>0.05$) between male and female mice. Similarly, the relative weights of these organs were not significantly different ($p>0.05$) in both sexes of mice at 3 weeks of age. Joubert (1956) reported that the size of any organ is dependent on the body size of the animal. Thus, the similarity in the relative weights of internal organs of male and female mice at 3 weeks of age may be related to the similarity in the body weights of these mice at this age. However, the heart of male mice was found to be significantly heavier ($p<0.05$) than that of female mice at 3 weeks of age. The reason for this exception is not well understood, but Balinsky (1970) suggested that the growth of different organs and of parts of the same animal seldom go on at the same rate. At puberty (6 weeks of age), the weights of all the internal organs studied were significantly greater ($p<0.01$) in males than females. This observation was consistent, even when the relative weights of these organs in males and females were statistically compared. Since the relative-organ-weight is an allometric index that yields a size independent dimensional constant, it allows comparison of organ weights of individuals of varying body weights (Stahl, 1965). It follows therefore that the weights of organs of male mice were indeed greater than those of female mice at 6 weeks of age. The sex difference cannot be attributed to the influence of body weight. The only exception was the lungs whose relative weight was not significantly different ($p>0.05$) between male and female mice at 6 weeks of age. At 12 weeks of age, the weights of heart, liver and kidneys remained greater in males than females, but sex differences were not observed in the weights of lungs and spleen. These variations in the growth patterns of internal organs of male and female mice were not unexpected. Balinsky (1970) had suggested that the growth of different organs and of parts of the same animal seldom go on at the same rate. Furthermore, the ultimate size of any organ is influenced by the function it performs (Goss, 1964). These factors may have given rise to the variations observed in the growth patterns of internal organs in both male and female mice.

In conclusion, this study demonstrated that there were sex differences in the growth patterns of skeletal muscles, bones and internal organs of mice. In most cases, the organs in the male were bigger than those

in the female. These observations suggest that sex hormones may exert significant influences on the growth of organs in male and female mice.

REFERENCES

ABERLE, E.D. and DOOLITTLE, D.F. (1976): Skeletal muscle cellularity in mice selected for large body size and controls. *Growth* **40**: 133 –145.

ARMSTRONG, D.T. and PAPKOFF, H. (1976): Stimulation of aromatization of exogenous androgens in ovaries of hypophysectomized rats *in vivo* by Follicle Stimulating Hormone. *Endocrinol.* **99**: 1144 –1151.

BALINSKY, B.I. (1970): An introduction to Embryology. Third Edition. W. B. Saunders Company. Philadelphia.

BENNET, J.P. and VICKERY, B.H. (1970): Puberty and development of gametes in rats. In: Reproduction and Breeding Techniques for Laboratory animals (Ed. Hafez E.S.E.) Lea and Febiger Philadelphia.

BURESOVA, M; GUTMAN, E. and KLIPERA, M. (1969): Effects of testosterone on the incorporation of amino acids into the proteins of levator ani muscle *in vitro.* Physiol. Bohemoslov. **18**: 137 –139

CHEEK, D.B.; BRASEL, J.A and GRAYSTONE, J.E. (1968): Muscle cell growth in rodents: sex differences and the role of hormones. In: Human growth, body composition, cell growth, energy and Intelligence. First Edition. (Ed. Check D.B) Lea and Febiger Philadelphia.

FISHER, R.A. and YATES, F. (1967): Statistical tables for biological, agricultural and medical research. Hafner New York.

FRIED, R.I. and SMITH, E.E. (1962): Post-menacheal growth patterns. *J. Pediat.* **61**: 562 – 565.

GOLDSPINK, G. (1972): Postembryonic growth and differentiation of striated muscle. In: G.H. Bourne (ed.) Structure and Function of Muscles. Second Edition. Acad. Press New York.

GOLDSPINK, G. and ROWE, R.W.D. (1968): The growth and development of muscle fibres in normal and dystrophic mice. In: Research in Muscular dystrophy. Proc. 4th Symposium on Current Research in Muscular Dystrophy. Pitman London.

GOSS, R.J. (1964): Adaptive growth. Logos Press Ltd. London.

IHEMELANDU, E.C. (1980): Effect of oestrogen on muscle development of female rabbits. *Acta. Anat.* **108**: 310 – 315.

IHEMELANDU, E.C. (1981): Comparison of effect of oestrogen on muscle development of male and female mice. *Acta Anat.* **110**: 311 – 317.

IHEMELANDU, E.C. (1984): Genesis of oestrogenic inhibition of soleus

muscle development in female mice. *Res. Vet. Sci.* **37**: 138 –140.

IHEMELANDU, E.C. (1988): Comparison of pre-pubertal muscle growth in male and female rats. *Zariya Vet.* **3**: 33 – 39.

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

JOUBERT, D.M. (1956): An analysis of factors influencing postnatal growth and development of muscle fibres. *J. Agric.* Sci. **47**: 59 –102.

MEARA, P.J. (1947): Postnatal growth and development of muscles as exemplified by the gastrocnemius and psoas muscles of rabbit. *Onderstepoort J. Vet. Sci. Anim. Ind.* **21**: 329 – 482.

RIESENFELD, A. (1976): Endocrine control of post-cranial body proportion. *Acta Anat.* **94**: 321 – 335.

ROWE, R.W.D. and GOLDSPINK, G. (1969): Muscle fibre growth in five different muscles in both sexes of mice I: Normal mice. *J. Anat.* **10**: 519 – 530.

STAHL, W.B. (1965): Organ weights in primates and other mammals. *Sci* **150**: 1039–1042.

VENABLE, J.H. (1966a): Constant cell populations in normal, testosterone – deprived and testosterone – stimulated levator ani muscles. *Amer, J. Anat.* **119**: 263–270.

VENABLE, J.H. (1966b): Morphology of cells of normal, testosterone-deprived and testosterone – stimulated levator ani muscles. *Amer. J. Anat.* **119**: 271–302.

WARREN, D.W.; HALTMEYER, G.C. and EIK-NES, K.B. (1975): The effect of gonadotrophins on the foetal and neonatal rat testes. *Endocrinol.* **96**: 1226–1229.

WETTENHALL, H.N.B. and ROCHE, A.F. (1965): Tall girls' assessment and management. *Aust. Pediat. J.* **1**: 210–216.