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Genesis of oestrogenic inhibition of soleus muscle development in female mice

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The mechanism by which oestrogen inhibits development of muscle mass was investigated in the soleus muscle of 20 sexually immature female mice. Half of the population were injected weekly (from one to nine weeks old) with a physiological dose of 0·01 mg stilboestrol dipropionate (synthetic oestrogen). After each mouse was killed at 10 weeks old, the weight and muscle index was determined for each muscle. The number of muscle fibres was counted in each soleus muscle. The muscle fibre size for each muscle was determined by measuring the greatest diameter of at least 100 fibres. The number of muscle fibres was similar in both normal and oestrogen treated animals but the individual muscle fibre sizes were significantly smaller in oestrogen treated animals. These observations indicated, therefore, that oestrogen inhibits development of muscle mass in females by limiting the size of individual muscle fibres and not by inhibiting the multiplication of muscle fibres.

THE muscles of the male mouse have been found to be invariably larger than those of the female (Rowe and Goldspink 1969, Aberle and Doolittle 1976) and in rats (Cheek et al 1968). This larger muscle mass of males could be attributed to the stimulatory effect of testosterone on muscle mass development. At the cellular level, Venable (1966) observed that replacement therapy using testosterone following castration of males caused considerable hypertrophy of the fibres in the rat anal muscles. Kochakian et al (1961) reported that the temporal and masseter muscles of the guinea pig were very sensitive to castration and subsequent testosterone replacement therapy. Buresova et al (1969) in their own investigation observed an increased incorporation of labelled amino acids into the muscle protein following administration of androgens to both intact and castrated animals. It has also been reported that ribosomes obtained from castrated male animals were less active in synthesising protein than those from intact animals (Breuer and Florini 1965). The ability of ribosomes to synthesise protein can also be modified by using a combination of testosterone and growth hormone (Florini and Breuer 1966). The smaller muscle mass of females, on the other hand, has been attributed to the effect of oestrogen (Cheek et al 1968, Ihemelandu 1980). Ihemelandu (1981) demonstrated that, in the presence of oestrogen, there were no differences between the muscle mass of developing male and female mice.

Ihemelandu (1980) made his observations at gross level following administration of physiological doses of oestrogen to developing female rabbits. Cheek et al (1968), on the other hand, made their deductions from observations in ovariectomised rats. According to the latter authors, ovariectomy produced slower growth rate in muscle fibre size and increased the number of muscle fibres. They interpreted this to mean that oestrogen inhibits multiplication of muscle fibres but stimulates growth in muscle figre size. They concluded, therefore, that males have larger muscles because they contain a greater number of muscle fibres. This observation of Cheek et al (1968) is contrary to that of Rowe and Goldspink (1969). These latter authors reported that the male possessed larger muscle mass because it had larger muscle fibres since the number of muscles fibres was similar in both sexes. Their observation implied that the effect of oestrogen is on the muscle fibre size. This means that oestrogen may limit muscle mass through inhibition of production of myofibrillar protein.

Aberle and Doolittle (1976) did not observe any differences between the numbers and sizes of muscle fibres in the soleus muscle of male and female mice even though they reported that muscle weights of males were significantly larger than those of the females. Their observation implied that oestrogen has no effect on either the number or the sizes of muscle fibres of soleus muscle and that the larger soleus muscle of males resulted from presence of greater non-muscle fibre materials.

While the direct effect of testosterone at the cellular level has been established to be stimulatory on the muscle fibre size, that of oestrogen needs further clarification. The purpose of this study was to investigate the direct effect of oestrogen on the

number and size of muscle fibres of developing soleus muscle in female mice through administration of a physiological dose of the hormone from one to nine weeks old. This period of study was chosen because oestrogen is produced normally in only small quantities from fetal life until onset of puberty (six to seven weeks old) when the production becomes accelerated. Secondly, Rowe and Goldspink (1969) have demonstrated that the muscles of female mice reach their mature weights at nine weeks old.

Materials and methods

Twenty female inbred mice were studied. From when the mice were one week old, 10 were injected intramuscularly (in the biceps femoris muscle) weekly with 0·01 mg of stilboestrol dipropionate (synthetic oestrogen). The injection site was alternated between the right and left hind limbs. Treatment was stopped after nine weeks old. The bodyweight of each mouse was determined just before each injection. The other 10 mice served as controls. They were similarly injected with 0·01 ml sterile olive oil, used as the vehicle for the oestrogen and their bodyweights determined weekly.

The mice were weaned at 21 days old. From that age the experimental groups were housed separately. They were all raised under the same environmental conditions and fed water and commercial mouse diet ad libitum. Each mouse was killed at 10 weeks old by severing the spinal cord at the occipito-atlantal joint. The final bodyweight was determined just before each mouse was killed.

The soleus muscle of the right limb of each mouse was used to determine the muscle weight. Each muscle was weighed immediately it was dissected out. The muscle mass index (mg muscle [g bodyweight]$^{-1}$) was also calculated for each muscle. The soleus muscle of the left limb of each mouse provided the materials for histological examinations. Soon after the muscles were dissected out they were fixed in formol saline (sodium chloride 0·9 per cent, formaldehyde 4 per cent) for 48 hours. At the end of the fixation period the muscles were placed in 10 per cent sulphuric acid for about two hours so as to produce slight separation of the fibres and facilitate counting (Rayne and Crawford 1975). The muscles were then dehydrated, cleared and embedded in paraffin wax. Transverse sections 8 μm thick were cut from the middle of the muscle belly in order to include all muscle fibres. The sections were stained with haematoxylin and eosin and mounted in Canada balsam.

Quantitative measurements

The total number of fibres was determined for each muscle by projecting the fibres on a screen of white cardboard using a micro-projector. An appropriate magnification was chosen so that all the fibres were projected on the screen at the same time. The fibres were counted directly using a colour marker to indicate a fibre counted.

The greatest diameter of each muscle fibre was determined by using the same micro-projector to project the fibres on a screen of white cardboard as above. The magnification chosen ensured that at least 100 fibres were projected fully within the field of view on the screen since it has been shown by other workers that it is statistically valid to use this size of sample and method of measurement (Meara 1947, Joubert 1956, Rowe and Goldspink 1969). An average of 109 fibres (102 to 131) were measured for each muscle.

The results obtained were statistically analysed. Analyses of variance (Student *t* test) was used to examine whether the differences between normal and oestrogen treated mice were statistically valid (Snedecor 1965, Fisher and Yates 1967).

TABLE 1: Comparison of different parameters of normal and oestrogen treated mice. Mean ± SE given for each measurement

Measurement	Normal mice	Oestrogen treated mice	Student *t* test	Degrees of freedom
Bodyweight (g)	27·44±0·83	27·08±1·14	P>0·05	18
Muscle weight (mg)	6·29±0·43	3·64±0·35	P<0·001	18
Muscle index (mg muscle [g bodyweight]$^{-1}$)	0·251±0·014	0·152±0·15	P<0·001	18
Number of muscle fibres	873±35	828±30	P>0·05	18
Muscle fibre size (μm)	38±2	30±2	P<0·001	18

Results

The data for all the parameters measured are presented in Table 1. The bodyweight of normal and oestrogen treated female mice were similar. The muscles of normal females were significantly heavier than those of oestrogen treated females (P<0·001). This was true for both the absolute and relative muscle weights (mg muscle [g bodyweight]$^{-1}$). The number of muscle fibres did not differ between normal and oestrogen treated animals. The muscle fibre diameter of normal animals was significantly greater than that of the oestrogen treated mice (P<0·001).

Discussion

It was observed from this study that the muscle

weight of oestrogen treated animals was significantly smaller than that of normal mice. This is similar to the observations in developing female rabbits (Ihemelandu 1980) and in male and female mice (Ihemelandu 1981) that oestrogen inhibits development of muscle mass.

Analyses of fibre numbers and sizes indicated that the number of fibres present in the soleus muscle of normal and oestrogen treated animals were similar. The fibre sizes, on the other hand, were greater in the soleus muscles of normal animals as compared to the oestrogen treated mice. These observations indicated that oestrogen inhibits muscle mass development by limiting the fibre sizes and not by inhibiting the multiplication of the number of muscle fibres. It does this possibly through inhibition of production of myofibrillar protein during the normal post natal muscle fibre hypertrophy. These observations are contrary to the suggestions of Cheek et al (1968) that oestrogen produces its inhibitory effect by reducing or preventing increase in the number of muscle fibres and also that oestrogen has a stimulatory effect on the muscle fibre sizes.

These contradictory results of Cheek et al (1968) may be due to the method they employed in determining the fibre numbers. Rayne and Crawford (1975) stated that the best way of estimating fibre numbers is to choose a muscle small enough that a section cut through its mid-belly will include all the fibres which can then be counted directly. They observed that wrong conclusions have been drawn from indirect fibre estimating methods which failed to take into account the internal architecture of the muscle fibre arrangement. In this study a section was cut through the mid-belly of the soleus muscle which included every fibre present in the muscle. These were then counted directly.

Rowe (1967) demonstrated that the fibres in the soleus muscle run from tendon of origin to tendon of insertion, hence a section through its mid-belly will include all the fibres. Cheek et al (1968) on the other hand used an indirect method of fibre estimation to determine the number of fibres present in the sample of muscles they analysed in the rats. Secondly they estimated the total number of fibres present in all the muscles of the rat pooled together. Obviously they did not take into account the internal architecture of the muscle fibre arrangement in the muscles they studied. Thirdly they neither studied individual muscles nor individual muscle fibres. As cited earlier it has been shown that for fibre sizes to be statistically valid the diameter of at least 100 fibres should be measured.

The size of muscle fibres may be influenced by species, breed, age, body size and weight, sex, nutrition, individual muscles and their functions (Joubert 1956). In this study both the normal and oestrogen treated female mice were fed ad libitum and raised under the same environmental conditions. The muscles from both the normal and oestrogen treated mice were processed for histological analyses in similar manners. Therefore, any effect of those factors on the muscle fibre size would presumably be the same for both the normal and oestrogen treated mice, hence the differences observed between groups in this study cannot be attributed to those factors.

In conclusion this study has demonstrated that oestrogen inhibits development of muscle mass in the female mouse by limiting the individual muscle fibre size and not by inhibiting the multiplication of the number of muscle fibres.

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