The mechanism of milk ejection in the lactating marsupial has, until relatively recently, been the subject of debate (Griffiths and Slater, 1988). Fundamental to the controversy has been the role played by the ilio-marsupialis muscle, which consists of skeletal muscle tissue originating on the pelvis and passing to the mammary area (see Fig. 1). Before it was known that the marsupial neonate was capable of sucking, it was postulated that voluntary contraction of the ilio-marsupialis muscle would compress the mammary glands and force milk through the nipples, hence the name M. compressor mammae was originally given to the M. ilio-marsupialis (see Griffiths and Slater, 1988, for discussion). The historical setting for this belief, and early observations on the ability of the young to suckle, have been documented by Hill and Hill (1955) and Enders (1966). Later workers (see Tyndale-Biscoe and Renfree, 1987) demonstrated that milk was not expressed from the glands by the contraction of skeletal muscle. It was suggested by Griffiths and Slater (1988) that the function of the ilio-marsupialis muscle was for mammary support and for retraction of the teats and attached young. This was considered by them to be especially important in ‘pouchless’ or ‘pseudo-
covered by a circular fold of skin with a central opening and all the nipples are enclosed. Young attached to the nipples are exposed throughout lactation in species with a Type 1 pouch but are covered, at least in the early stages, in species with a Type 3 pouch.

In the present study, observations have been made on the ilio-marsupialis muscles of *Sminthopsis douglasi*, a dasyurid marsupial with a Type 3 pouch. There are eight nipples in the pouch of this species and this determines the maximum litter size because the young do not relinquish the nipple until approximately day 40 of the 70-day suckling period. In the event that the female does not have a full complement of young, the mammary glands associated with unsuckled nipples do not enlarge and produce milk. For approximately 30 days, the young are covered by the pouch skin fold, after which they become progressively more exposed (Fig. 2). By 40 days of age, they hang out of the pouch but are usually held close to the body of the mother when she is moving around. From this age, they begin to be left in the nest while the mother is out feeding (P. A. Woolley, unpublished laboratory observations). *Sminthopsis douglasi* is nocturnal and insectivorous but little is known of its activity patterns in the wild. It would seem to be advantageous for the mother to be able to forage without the burden of large young. The mechanism by which the mother dislodges the young is not known, but young in captivity have been observed to relinquish the nipples following repeated rapid movements (vibration) of the abdomen (P. A. Woolley and M. Coleman, unpublished observations).

From this brief description of events in the development of the suckling young, it seems likely that the demands placed on the ilio-marsupialis muscle may change over the suckling period. As mammalian skeletal muscles in general display plasticity (Pette and Staron, 1997, 2001; Pette, 1998), changing demands on the ilio-marsupialis muscle would be expected to induce changes in the fibre-type composition. Therefore, bearing in mind that dynamic muscular activity is usually associated with fast-twitch muscle fibres, and postural-type activity is usually associated with slow-twitch, fatigue-resistant muscle fibres (Jones and Round, 1990), it was of interest to investigate whether or not changes occurred in the type of muscle fibres present in the ilio-marsupialis muscles of *Sminthopsis douglasi* in the course of the development of suckling young. Identification of such changes might also help to better understand the function of this muscle.

**Materials and methods**

**Animals**

Ten females bred in captivity were obtained from a colony of *Sminthopsis douglasi* Archer 1979 kept at La Trobe University (Woolley, 1995). Eight of the females were suckling young. Young less than 40 days of age were removed from the nipples and formed part of a separate study on growth and development, while those older than 40 days were fostered to other mothers. The adult females were killed by gaseous anaesthetic overdose, and a midline incision was made through the pouch and abdominal skin to expose the mammary tissue and ilio-marsupialis muscles. Branches of the muscles supplying particular nipples were identified, and 3–5 mm lengths were removed, blotted dry on filter paper and then placed under paraffin oil on a base of Sylgard 184 (Dow Chemicals, Midland, MI, USA). Some of the lactating females did not have a full complement of young and so muscles...
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associated with both suckled and unsuckled nipples were obtained from most of these individuals. One half of the pouch area (skin, mammary glands and muscles) from each of two individuals was fixed in Bouin’s solution (glacial acetic acid, 40% formaldehyde, saturated aqueous picric acid, 1:5:15), and serial 10 μm paraffin sections were prepared. Alternate slides were stained with Haematoxylin and Eosin, and Haematoxylin, Xyloidene Red and light green SF (Masson’s trichrome method). Carcasses were preserved for later dissection to establish the point of insertion of the ilio-marsupialis muscles on the pelvis.

**Fibre typing**

It has been shown that muscle fibres from eutherian mammals (Fink et al., 1990; Lynch et al., 1995) and dasyurid marsupials (Wilson and Stephenson, 1990) can be classified according to their sensitivity to the activators strontium (Sr²⁺) and calcium (Ca²⁺) into three groups: fast-twitch, slow-twitch and composite. Compared with slow-twitch fibres, fast-twitch fibres display a greatly reduced sensitivity to activation by Sr²⁺ ions and a slightly decreased sensitivity to activation by Ca²⁺ ions. Composite fibres display high Sr²⁺ sensitivity at lower levels of activation (similar to slow-twitch fibres) but low Sr²⁺ sensitivity at higher levels of activation (similar to fast-twitch fibres). This physiological method of fibre classification has been validated previously by an electrophoretic method for the determination of the myosin heavy chains in the same fibres for which activation by Sr²⁺ and Ca²⁺ was measured (Bortolotto et al., 2000).

Between two and nine fibres per animal were examined. Single fibres were randomly isolated from freshly dissected branches of the ilio-marsupialis muscle associated with a particular nipple, and their Sr²⁺ and Ca²⁺ activation characteristics were determined after brief treatment with Triton X-100, a non-ionic detergent, to remove the membrane barriers and enable access to the contractile apparatus (Bortolotto et al., 2000). The fibres were then activated in Sr²⁺- and Ca²⁺-buffered solutions and classified as fast, slow or composite, and the proportions of the different types of fibres were calculated. This method has been shown previously to produce a fibre-type profile that is very close to that obtained from the sampling of the entire muscle (Bortolotto et al., 2000).

Samples of the ilio-marsupialis muscle associated with individual nipples were taken from non-lactating and lactating females and grouped as follows:

- Group 1 – nipple never suckled (two non-parous individuals),
- Group 2 – nipple being continuously suckled (three individuals with young of 35 days old, 36 days old and 39 days old, respectively),
- Group 3 – suckling intermittent (three individuals with young of 51 days old, 52 days old and 53 days old, respectively),
- Group 4 – late lactation (weaning), (two individuals with young of 68 days old and 70 days old, respectively),
- Group 5 – unsuckled nipple in the pouch of a female with young suckling other nipples (four individuals with young of 36 days old, 39 days old, 52 days old and 53 days old, respectively).

The mass of the young, and of the mammary tissue, was obtained for some of the above females and for others at particular times throughout the lactation period as part of a separate study.

The proportions of the three types of fibres present in the ilio-marsupialis muscles in non-lactating females, and in lactating females at different stages of lactation, were analysed by Fisher exact test. All statistical analyses were two-tailed, and significance was accepted at the \( P<0.05 \) level.

**Results**

**Morphology**

The ilio-marsupialis muscle of each side of the body originates on the anterior iliac spine and is closely associated

![Image](image-url)
with the lumbar musculature before passing through the inguinal canal to lie superficial to the abdominal musculature dorsal to the pouch area (Fig. 1). There, each muscle divides into four branches, each of which can be traced to the base of one of the four nipples on the corresponding side of the pouch. In serial histological sections, each branch can be seen to fan out from beneath the nipple and insert on to the skin of the pouch and that of the nipple itself. Strands of ilio-marsupialis muscle can be seen in a section through a nipple and the adjacent pouch skin in Fig. 3. There is an obvious increase in the size of the muscle and of its branches to suckled nipples during lactation, but this increase is difficult to quantify because the muscle cannot be dissected from tendon to tendon.

Muscle fibre-type profiles

Fig. 4 shows the stages at which the groups of muscle samples were taken and the appearance of the pouch together with, in females suckling young, major events during the suckling period. The number of fibres examined and the number of animals they were obtained from, together with the average percentage of fibre type per animal ($f_A$) and the percentage of fibre type per total fibres ($f_T$) in each of the five groups are given in Table 1. It is important to note that, despite the variation in the number of fibres investigated per animal (2–9), the average percentage of $f_A$ was close to the fraction of $f_T$ examined for each group. This indicates that the inter-animal variability in the fibre composition of the ilio-marsupialis muscle is relatively small. The largest difference between $f_A$ and $f_T$ values was found in Group 5 (unsuckled nipples), where fibres were taken from animals at very different stages of lactation. This may account for the higher inter-animal variability. Nevertheless, even in this group, there was consistency in the sense that no slow-twitch fibres were found in any of the four animals examined. Given the close values between $f_T$ and $f_A$ in Table 1, only the $f_T$ values for the fast-twitch, slow-twitch and composite fibres are presented in Fig. 5.

In Group 1, the majority of fibres (71%) were classified as composite, with only 22% showing fast and 7% showing slow activation characteristics. The fibre-type profile of this group represents the steady-state (control) condition of the ilio-marsupialis muscle in females not suckling young. In muscles associated with suckled nipples (Groups 2, 3 and 4), the proportion of composite fibres was less than in Group 1. In Group 2, there was a greater proportion of fast fibres (75% versus 22%, $P<0.05$) and a lower proportion of composite fibres (25% versus 71%, $P<0.05$) than in Group 1. Further shifts in fibre-type composition were seen in Groups 3 and 4. In Group 3, there was a significant increase in the proportion of slow fibres (39% versus 0%, $P<0.05$) and, consequently, a lower proportion of fast fibres (39% versus 75%, $P<0.05$) than in Group 2. In Group 4, when weaning of the young is in progress, the proportion of composite fibres was lowest (6%), with most fibres being classified as either fast (69%) or slow (25%). These results show that the principal effect on the ilio-marsupialis muscle during progression of the lactation period is the transformation of composite fibres associated with suckled nipples into either fast-twitch or slow-twitch fibres. In our view, the fibre type transformation in both directions is not caused by one stimulus, i.e. suckling, but rather by changes in the load placed on the ilio-marsupialis muscle associated with growth of the young and changes in their behaviour (see Discussion). The change first seen was from composite to fast-twitch fibres and, later in the lactation period, it was also to slow-twitch fibres. Fibres in Group 5 (associated with unsuckled nipples in the pouch of females with young suckling other nipples) showed a similar fibre-type profile to those in Group 1 (Fig. 5). No significant difference ($P=0.39$) was found between the types of fibres identified in Groups 1 and 5, which suggests that
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The ilio-marsupialis muscle in dasyurid marsupials has no effect on fibre-type profile; rather, it is the demand on the muscles associated with individual nipples that is related to changes in fibre-type profile.

Discussion

It is accepted that mammalian skeletal muscle is a dynamic tissue that undergoes adaptive changes in response to altered functional demands (Pette and Staron, 1997, 2001; Pette, 1998). Muscle tissue is therefore not composed of fixed percentages of different types of fibres but instead exhibits fibre-type plasticity (or adaptability) that takes place in response to altered physical demands placed on the muscle. Numerous studies have demonstrated such plasticity of the muscle fibres. For example, fast-to-slow and slow-to-fast transitions in myosin heavy chain isoforms have been shown to occur in response to altered neuromuscular activity resulting from chronic low-frequency stimulation (Leeuw and Pette, 1993), endurance training (e.g. Klitgaard et al., 1990) and hindlimb suspension (Stevens et al., 2000). In the present study, we present evidence that the fibre-type profile of the branches of the ilio-marsupialis muscle in the marsupial Sminthopsis douglasi changes when the nipples are suckled and in a manner dependent on the age of the suckling young.

In the absence of suckling young, the ilio-marsupialis muscle consists predominantly of composite fibres (71%). By comparison, only 28% of the fibres from the soleus muscle of the stripe-faced dunnart Sminthopsis macroura, a smaller congener of the species used in this study, were composite (Wilson and Stephenson, 1990). A high proportion of composite fibres in a muscle normally indicates a more complex functional role of that muscle (Stephenson, 2001). When the nipples are not suckled, the branches of the muscles are small, suggesting little or no functional role other than perhaps to maintain the tone of the pouch skin fold. The high proportion of composite fibres in these muscles would enable them to adapt to changes in demand during the suckling period.

In the presence of suckling young, the fibre-type profile of the ilio-marsupialis muscle changed. For approximately the first 40 days of the lactation period, the young are suckled continuously (the attachment phase), and during this time lactation per se has no effect on fibre-type profile; rather, it is the demand on the muscles associated with individual nipples that is related to changes in fibre-type profile.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. fibres (no. animals)</th>
<th>Fast-twitch</th>
<th>Composite</th>
<th>Slow-twitch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% $f_A$</td>
<td>% $f_T$ %</td>
<td>% $f_A$ % $f_T$</td>
</tr>
<tr>
<td>1</td>
<td>14 (2)</td>
<td>21</td>
<td>22</td>
<td>73</td>
</tr>
<tr>
<td>2</td>
<td>12 (3)</td>
<td>72</td>
<td>75</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>18 (3)</td>
<td>37</td>
<td>39</td>
<td>19</td>
</tr>
<tr>
<td>4</td>
<td>16 (2)</td>
<td>69</td>
<td>69</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>17 (4)</td>
<td>52</td>
<td>41</td>
<td>48</td>
</tr>
</tbody>
</table>

Table 1. The number of fibres, the number of animals, the average percentage of fibre type per animal ($f_A$) and the percentage of fibre type per total fibres ($f_T$) in each group examined.

Group 1, nipples never suckled; Group 2, nipples suckled for 36–39 days; Group 3, nipples suckled for 51–53 days; Group 4, nipples suckled for 68–70 days; Group 5, unsuckled nipples in the pouch of females with young on other nipples.

Fig. 4. The groups of ilio-marsupialis muscle samples taken from the animals used in this study are shown in relation to the schematic appearance of the pouch in both non-suckling adults and in females suckling young, together with events during the suckling period. Arrows indicate the ages of the young in the samples taken from lactating females. Group 1, nipples never suckled; Group 2, nipples suckled for 36–39 days; Group 3, nipples suckled for 51–53 days; Group 4, nipples suckled for 68–70 days; Group 5, unsuckled nipples in the pouch of females with young on other nipples.
Growth of the young accelerates after day 40, with 51–53-day-old young (Group 3) each weighing as much as 6 g, and 68–70-day-old young (Group 4) weighing 15–20 g. These older young are not carried in the pouch when the mother is out foraging, but there is an increase in the mass of mammary tissue up to approximately 10 g (15–20% of maternal body mass at the beginning of lactation). The most pronounced change in fibre-type composition of the ilio-marsupialis muscles associated with suckled nipples in Groups 3 and 4 is an increase in the proportion of slow-twitch (fatigue-resistant) fibres. This may occur in response to the need to support a greater mass of mammary tissue and, although there may be little need to hold the young close to her, at least some of the mass of the young while they are suckling in the nest. Studies on captive females with young of these ages have shown that the young will relinquish the nipples following vibration, for 5–60 s, of the mother’s abdominal muscles and possibly also the ilio-marsupialis muscles. The major difference between fibre-type profiles in the ilio-marsupialis muscles of Groups 3 and 4 is the transformation of composite (uncommitted) fibres into fast-twitch fibres. The presence of a high proportion of fast-twitch fibres supports a dynamic function for them at these stages.

Griffiths and Slater (1988) considered that the role of the ilio-marsupialis muscle in the ‘pouchless’ dasyurid marsupials that they examined was to haul the young up tightly into the pouch area and so protect them from injury. In Antechinus stuartii (the species in which they illustrated that anaesthesia reduced the ability of the mother to hold her young close to the body) and other ‘pouchless’ dasyurids, the young are exposed (i.e. not enclosed by pouch skin) from the time they are born. It would be of interest to examine the fibre-type composition of the ilio-marsupialis muscles of ‘pouchless’ species to determine what changes occur throughout the suckling period for comparison with species such as S. douglasi. Such a study might help to provide a better understanding of the function of the ilio-marsupialis muscles in ‘pouched’ and ‘pouchless’ marsupials.

These experiments were carried out with approval from the La Trobe University Animal Ethics Committee (AEC # LSB 96/26). We thank Dr Alan Wright for photomicrography, Mr Greg Mifsud for use of the photograph of S. douglasi, Mr Trevor Phillips for preparation of figures, and Professor Ian Thornton for critical comments on the manuscript. This work was supported in part by the Australian Research Council and the Living Together Foundation.

Fig. 5. The relative proportions of fast-twitch, composite and slow-twitch muscle fibres in populations of fibres (ft) dissected from muscles associated with the nipples in each of the five groups studied. Group 1, nipples never suckled; Group 2, nipples suckled for 36–39 days; Group 3, nipples suckled for 51–53 days; Group 4, nipples suckled for 68–70 days; Group 5, unsuckled nipples in the pouch of females with young on other nipples.

References


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