# Stereological Evaluation of the Soleus Muscle Isografted into Fast Extensor Digitorum Longus (EDL) Muscle in Rats with Different Thyroid Status

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**Abstract.** The 2-D stereology can be used advantagenously in the case of muscle cross sections stained by routine histochemical and immunocytochemical methods, such as mATPase reaction, when the quality of the image is often not sufficient for using image analysis techniques without considerable individual intervention Other advantages of stereological methods in muscle morphometry are that measurements are made directly on specimens under the microscope and in their simplest arrangement they do not require sophisticated and expensive technical equipment Furthermore, unbiased results are obtained, no segmentation and edge effect problems arise and the quantity of work invested in stereological estimation is reasonable Therefore, we have used the stereological methods as our standard technique for assessment of fibre type composition in regenerated soleus muscles grafted from 21- to 28-day-old rats into fast EDL muscles of adult inbred recipients with different plasma levels of thyroid hormones

**Key words:** Muscle transplantation — Fibre types — Regeneration — Stereology — Thyroxine

The structural and functional diversity of skeletal muscle reflects a variety of myosin isoforms Slow-twitch type 1 fibres and fast-twitch type 2 fibres are known to contain different myosin heavy chain (MyHC) isoforms that are responsible for their different ATPase activities and speed of contraction, in adult mammalian skeletal muscle, three subpopulations of type 2 skeletal muscle fibres, type 2A, 2B and 2X/D, have been described and three corresponding fast type MyHC isoforms 2a, 2b and 2x/d have been identified, developing and regenerating muscle fibres express embryonic and neonatal MyHC isoforms (for review, see Schiaffino and Reggiani 1994, Pette and Staron 1997) Other MyHC isoforms, slow tonic, extraocular, superfast and alfa cardiac are as a rule not expressed in extrafusal muscle fibres, but they do occur in highly specialized extraocular and masticatory or the tensor tympani muscles and in intrafusal fibres of muscle spindles (for review, see Soukup *et al* 1995, Pette and Staron 1997)

Mammalian skeletal muscles consist of heterogeneous populations of physiologically and biochemically diverse fibres, their ratio differs from muscle to muscle reflecting the function and usage of different muscles Muscle fibre phenotype is the result of an interaction between factors intrinsic to the myoblast lineage from which myofibres develop and

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extrinsic factors such as type of innervation, muscle contractile activity, level of thyroid hormones and stretch conditions (Miller and Stockdale 1986, Gunning and Hardeman 1991, Pette and Staron 1997) Skeletal muscles react to alterations of individual extrinsic factors by changing the phenotype of their fibres Muscle injury and regeneration make a muscle more susceptible to transformation, regenerated rat fast muscle transplanted to the slow muscle bed and innervated by the slow nerve, exhibits an identical MyHC pattern to that of slow muscles (Snoj-Cvetko *et al* 1996) Our interest was to learn how the grafted and regenerating muscles will be altered by cross-innervation in different thyroid states, which are known to affect the expression of muscle fibre phenotype (Gambke *et al* 1983, Izumo *et al* 1986, Fitzsimons *et al* 1990, for review, see Pette and Staron 1997)

In our experiments, we grafted intramuscularly soleus muscles of 21- to 28-day-old rats into the fast EDL muscles in young adult euthyroid, hyperthyroid and hypothyroid inbred rats using a method described earlier (Jirmanová and Soukup 1995) Their mATPase reaction and expression of MyHC isoforms were studied 1 to 7 months after the operation (for methods see Soukup and Thornell 1997) The hypothyroid state was induced and maintained using a 0 05% solution of methimazole (Sigma) in the drinking water, while the hyperthyroid state was induced and maintained by intraperitoneal injections of DL-thyroxine in the dose 15 $\mu$g per 100 g body weight, 3 times a week for 6–15 weeks

When the grafted soleus muscle had been reinnervated by the fast EDL nerve in euthyroid rats, regenerated SOL transplants became comparable to the control EDL muscle already 7 weeks after the transplantation, type 2 fast fibres predominated in the graft and fast MyHC 2 isoforms were present in $\sim$ 85% of all muscle fibres (Fig 1 – Euthyroid rats) In hyperthyroid rats, the transformation of the SOL muscle was further enhanced, as $\sim$ 98% were of the fast type 2 fibres (Fig 1 – Hyperthyroid rats) In hypothyroid rats,

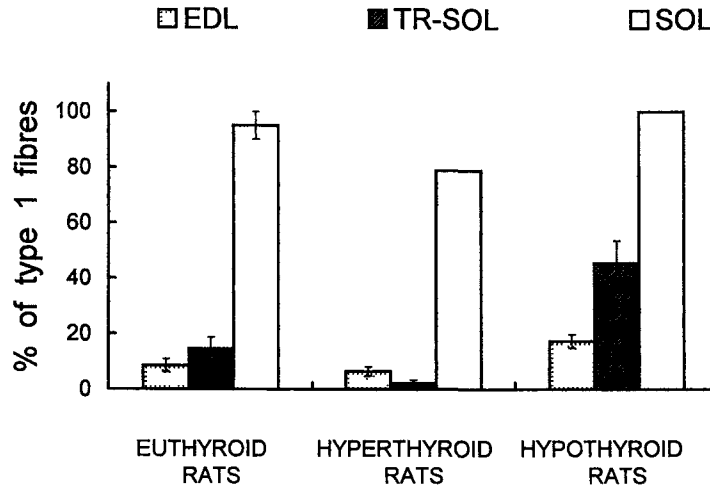


**Figure 1.** Percentage of type 1 fibres in regenerated soleus muscle (TR SOL) 7–29 weeks after transplantation compared with host EDL (EDL) and contralateral soleus (SOL) muscle in euthyroid ($n$ = 5), hyperthyroid ($n$ = 3) and hypothyroid ($n$ = 8) rats Vertical bars represent standard error of mean (SEM) SEM in SOL of hypothyroid rats is zero

body growth was practically arrested, the plasma levels of both triiodothyronine and thyroxine and of liver mitochondrial alpha-glycerol phosphate cytochrome C reductase were considerably reduced and the transformation of the slow soleus muscle was less pronounced than that in euthyroid rats (Fig 1 Hypothyroid rats), only 42% of regenerated soleus muscle fibres after 7 weeks, 62% after 12 weeks and 69% after 21–29 weeks post grafting were of the fast type 2 as shown by the mATPase reaction and MyHC expression The transformation of the slow soleus into fast EDL muscle type was thus slowed down in hypothyroid rats Interestingly, the hypothyroid state also increased the occurrence of intrafusal fibres with slow type 1 characteristics in regenerated muscle spindles (14%) in comparison to euthyroid (5%) or hyperthyroid (5%) state

Our results show that the normal expression of the fast type 2 phenotype in the regenerated soleus muscle grafted intramuscularly into the fast EDL muscle and innervated by the peroneal nerve supplying the recipient EDL muscle requires the concomitant contribution of normal levels of thyroid hormones It can therefore be concluded that normal levels of thyroid hormones are required for the expression of the fast type 2 phenotype accessible in the regenerated SOL muscle following reinnervation by the fast EDL nerve

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