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Postnatal changes in insulin binding in slow and fast-twitch rabbit skeletal muscles

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Summary. Changes of insulin binding characteristics (number of receptors, Kd) were studied in the semimembranosus proprius (SMP) and psoas major (PM) muscle from birth to adult stage. The Kd was about $0.15 \cdot 10^{-9}$ M in both muscles and did not change with age. The numbers of receptors were similar in both muscles at birth and changed differently thereafter to reach 1.3 fmoles per mg of muscle in the PM and 5.6 in the SMP muscle.

Introduction. — During postnatal development, histochemical and biochemical characteristics of mammalian skeletal muscles change markedly (Cooper *et al.*, 1970). This leads to different types of myofibers generally classified as slow-twitch oxydative (SO), fast-twitch oxydo-glycolytic (FOG) and fast-twitch glycolytic (FG) (Peter *et al.*, 1972). Because of the action of insulin on energetic and proteic metabolisms in the muscle (James *et al.*, 1985), it is of interest to determine whether insulin binding characteristics (number of receptors, Kd) are related to postnatal muscle fiber differentiation.

Material and Methods. — New Zealand white female rabbits from birth to the adult stage were used. Immediately after exsanguination at about 10hr, the semimembranosus proprius (SMP) and psoas major (PM) muscles, respectively SO and FG in the adult, were removed and quickly frozen in isopentane cooled with dry ice. The insulin binding characteristics were determined on 40 μ m thick muscle slices according to the method described by Lefaucheur *et al.* (1986). Four to five mg of muscle slices were incubated for 24 hours at 4 °C with $4 \cdot 10^{-11}$ M 125 I-insulin and various concentrations of unlabeled insulin. Results were analysed according to the Scatchard method, taking into account the high affinity binding sites only. The number of sites were expressed as femtomoles of specifically bound insulin per mg of fresh muscle.

Results and Discussion. — The Scatchard plots were curvilinear as previously reported for insulin receptor interaction (Kahn *et al.*, 1974). The high affinity constant (Kd) was about $0.15 \cdot 10^{-9}$ M in both muscles and did not change with

age. On the contrary, the number of high-affinity insulin receptors per mg of fresh muscle was similar in both muscles at birth (5.3 fmoles) but changed markedly thereafter and differently according to the muscle (fig. 1). In the PM muscle, it decreased immediately after birth and reached a minimum value of 0.2 fmoles at about 1.2 kg of body weight (BW). It then increased slightly to reach the adult value of 1.3 fmoles from 2 kg BW onwards. In the SMP muscle, the high-affinity insulin receptor concentration remained unchanged until 0.6 kg BW and then dramatically decreased to a minimum value of 0.9 fmoles around 1.2 kg BW. It subsequently increased to 3.7 fmoles at 2 kg BW and reached a plateau, approximately 5.6 fmoles, in the adult animal.

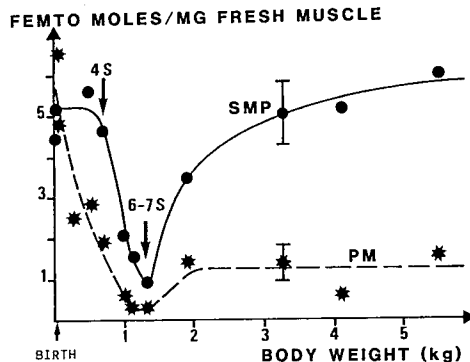


FIG. 1. — Postnatal changes in the number of insulin receptors per mg fresh muscle in the *semimembranosus* (●) and *psoas major* (★) muscle. Depending on the muscle weight, several muscles from different animals of the same weight and age had to be pooled to harvest a sufficient amount of muscle slices. Vertical bars indicate standard deviation ($n = 4$).

The difference of insulin binding in the adult muscles might be related to the more intense energetic and proteic metabolisms of SO fibers (James *et al.*, 1985) ; this is in accordance with the higher quantity of work they produce *in vivo* (Vollestad *et al.*, 1985). Postnatal changes in insulin receptor concentration could be linked to the metabolic differentiation of the myofibers. All fibers are indeed of the oxidative type in both muscles at birth. They convert then to the glycolytic type in the PM while they remain of the oxidative type in the SMP muscle. The marked decrease of receptor concentration at about 1.2 kg BW could be due to weaning, but further investigation is needed.

In *conclusion*, the present data show that the insulin receptor concentration changes postnatally and in a different way according to the myofiber composition. No change in affinity seemed to occur.

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