Myosin subunit composition in human developing muscle

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Previous pyrophosphate-gel studies have reported the existence of embryonic neonatal myosin isoenzymes in human developing muscle. The present investigation was undertaken to characterize their subunit composition more precisely. Two immature muscle myosins are contrasted with adult myosin: neonatal myosin and foetal myosin. The neonatal form of myosin is weakly cross-reactive with rabbit slow myosin and contains only fast-type light chains (LC), LC1F and LC2F. The associated heavy chains consist of a single electrophoretic component that reacts exclusively with antibodies against human foetal myosin and has a mobility and peptide pattern distinct from that of adult fast and slow heavy chains. Foetal myosin is distinguished by the presence of low amounts of a heavy chain immunologically cross-reactive with the adult slow form and of two additional light-chain components: a LC2S light chain and a foetal-specific light chain (LC_{emb.}). The foetal-specific light chain, as shown by one-dimensional-peptide-map analysis, is structurally unrelated to both LC1S and LC1F light chains of human adult myosin. We conclude from these results that the ontogenesis of human muscle myosin shares certain common features with that observed in other species, except for the persistence until birth of a foetal form of heavy chain (HC_{emb.}).

Knowledge of the ontogenic transitions of myosin heavy-chain isoenzymes in human skeletal muscle lags behind that obtained in other mammalian species, for which the existence of embryonic/foetal, neonatal, adult slow and adult fast isomyosins is now well documented (Whalen *et al.*, 1981; Lowey *et al.*, 1983; Caplan *et al.*, 1983). The evidence for myosin heterogeneity with respect to the heavy chains and at various stages of muscle development, comes mainly from electrophoretic studies of human native myosin in pyrophosphate gels (Fitzsimons & Hoh, 1981a). On the other hand, the nature of the associated light chains in the myosin of immature human muscle has been investigated in greater detail. We have shown (Volpe *et al.*, 1981, 1982), and this has been

confirmed recently by Strohman *et al.* (1983), that the two main light chains in human foetal myosin are those predominant in pure adult fast myosin, similar to other species (see Lowey *et al.*, 1983). A minor light-chain component, specific to human foetal myosin, has also been identified and tentatively classified as a slow type of light chain (Strohman *et al.*, 1983), possibly related to the LC1 light chain of human foetal or adult atrium and foetal ventricle (Cummins, 1983).

We report here on the characterization of the heavy chains of human foetal and neonatal myosin by direct electrophoresis in SDS/polyacrylamide gels, peptide-mapping analysis and, as a complementary approach, by specific antibodies using e.l.i.s.a., and Western-blot immunoenzymic-staining techniques. Furthermore, we show that the human foetal-specific myosin light chain is structurally and antigenically different from the LC1 light-chain components of adult myosin.

Parts of this work have been presented at an International Symposium on Muscular Dystrophy held in Modena, Italy, in 1983 (Margreth *et al.*, 1984).

Abbreviations used: LC, (myosin) light chain; SDS, sodium dodecyl sulphate; e.l.i.s.a., enzyme-linked immunosorbent assay; HC, (myosin) heavy chain; phosphate-buffered saline, 0.1M-sodium phosphate/0.15M-NaCl, pH 7.2; LC_{emb.}, foetal-specific light chain.

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Materials and methods

Tissue sources

Adult muscle biopsy specimens (pectoralis minor, vastus lateralis and vastus medialis) were obtained from 40–60-year-old female patients undergoing surgery. Foetal muscles (total thigh muscles) were from three 15–18-week-old human fetuses. Neonatal muscles were obtained as follows: vastus lateralis from an infant who died a day after birth (normal neonatal muscle); quadriceps femoris from an infant born prematurely (31 weeks gestation) and who died 5 days after birth of bronchopneumonia (immature neonatal muscle); psoas from an infant who was born anencephalic and died a day after birth with evidence on autopsy of defective formation of the forebrain (anencephalic neonatal muscle).

Preparative procedures

Myosin was prepared from human skeletal muscles by the method of Barany & Close (1971), as described previously (Volpe *et al.*, 1981), in the presence of pepstatin (200 µg/litre) in the extraction and purification media. Myosins were stored in 5 mM-Tris/HCl buffer (pH 7.6)/25 mM-KCl/0.05 mM-dithiothreitol/50% glycerol at -20°C , unless immediately used.

Electrophoresis

SDS/polyacrylamide-gel electrophoresis was carried out by using Laemmli's (1970) method in 0.75 mm-thick 5% or 15% (w/v)-polyacrylamide slab gels. Two-dimensional electrophoresis was carried out by the method of O'Farrell (1975), under conditions identical with those reported by Volpe *et al.* (1981). One-dimensional peptide maps of myosin heavy and light chains were performed under the general conditions of Cleveland *et al.* (1977), or with the modifications of Carraro *et al.* (1981), after partial digestion with *Staphylococcus aureus* V8 proteinase, in a 1 mm-thick 15–20% polyacrylamide-linear-gradient slab gel. Slab gels were stained with either Coomassie Blue or by the silver nitrate method described by Merrill *et al.* (1981).

Preparation of antisera and purification of antibodies

Affinity-purified antibodies to rabbit LC1F were the same as those previously characterized (Volpe *et al.*, 1981; Biral *et al.*, 1982).

Antisera to purified human foetal myosin were raised in adult hens by three intramuscular injections at 2-week intervals of 1 mg of antigen emulsified with an equal volume of complete Freund's adjuvant. Antibodies were purified by affinity chromatography as described by Volpe *et*

al. (1981). The batch of antibodies used in the present study was from a single bleeding.

The preparation of antisera and purification of antibodies against purified rabbit soleus myosin followed procedures identical with those reported above for human foetal myosin.

Immunological techniques

(1) *E.l.i.s.a.* One-step non-competitive and two-step competitive e.l.i.s.a. immunoassays were carried out under conditions identical with those described by Biral *et al.* (1982).

(2) *Western-blot immunoenzymic-staining techniques.* The electrophoretic transfer of proteins from Laemmli slab gels on to nitrocellulose sheets was carried out essentially as reported by Towbin *et al.* (1979), in 25 mM-Tris/HCl (pH 8.3)/192 mM-glycine/20% (v/v) methanol for 3½ h at 500 mA. The nitrocellulose sheets were first soaked with 3% bovine serum albumin in 150 mM-NaCl/0.1% Tween 20 for 1 h at 37°C , and were then incubated for 5 h at 37°C with antibodies diluted in phosphate-buffered saline (pH 7.2)/0.05% Tween 20/0.02% NaN_3 . The sheets were subsequently washed with 150 mM-NaCl/0.1% Tween 20 four times for 5 min and then re-incubated with anti-(chicken IgG) antibody, conjugated to alkaline phosphatase, diluted 1:50 (v/v) in phosphate-buffered saline (pH 7.2)/0.05% Tween 20/0.02% NaN_3 , for 1½ h at 37°C . After washing as above, the blots were stained by incubating at room temperature in either 50 mM-diethylaminopropanediol buffer, pH 9.8, containing Fast Blue BB salt (1 mg/ml) and α -naphthyl phosphate (1 mg/ml) (Procedure A) or in 97 mM-diethylbarbituric acid sodium salt/1.6 mM- MgSO_4 /Fast Blue BB salt (1 mg/ml)/ α -naphthyl phosphate (0.4 mg/ml) (Procedure B). The reaction was stopped after 15 min by washing with distilled water.

Determination of protein concentration

Protein concentration was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Results

(a) Heavy-chain complement of human foetal, neonatal and adult myosins

The heavy chains of myosin from foetal (15–18 weeks gestation) muscle, immature (31 weeks gestation) and at term (normal gestational period), neonatal (1-day) muscle and adult vastus lateralis muscle, were analysed by direct electrophoresis in SDS/5%-polyacrylamide slab gels in a Laemmli system. This method has already been used to advantage to separate the heavy chains of fast and slow myosins of adult chicken (Rushbrook

& Stracher, 1979) and rat (Carraro & Catani, 1983) muscle.

The presence of two components in the heavy-chain region of electrophoretograms of the myosin of human adult mixed muscle (Fig. 1), and the greater prominence of the slower-moving band in vastus lateralis, a predominantly fast muscle (Volpe *et al.*, 1981), are in agreement with the results of Carraro & Catani (1983). In particular, differences in relative mobility between adult fast and slow forms were observed. Neither of these components was detectable in human neonatal myosin, which contained instead a single heavy-chain form of intermediate mobility (Fig. 1). On the other hand, foetal myosin, as well as myosin from a premature infant, were contradistinguished by the presence of trace amounts of an additional heavy-chain component that co-migrated with the faster-moving heavy-chain band of adult myosin. Interestingly, this band was found to be much more pronounced in electrophoretograms of muscle myosin from an anencephalic infant (Fig. 1).

Immunoenzymic staining of blots of the electrophoretic gels with antibodies against rabbit slow myosin (Fig. 2) showed that the faster-moving electrophoretic band, but no other heavy-chain band, was cross-reactive, irrespective of the myosin source. In further experiments the whole myosin was probed with these antibodies by e.l.i.s.a. techniques. It was found that, in a range of antibody concentrations of 0.1–10 µg of protein, and at a fixed coating concentration of antigen (5 µg/ml), adult and anencephalic neonatal myosin were the most reactive, normal neonatal myosin the weakest, and foetal myosin of intermediate reactivity. Thus both lines of evidence supported the conclusion that foetal myosin, but not normal

neonatal myosin, contains a slow heavy-chain component.

Peptide mapping of myosin heavy chains was done by the procedure of Cleveland *et al.* (1977), after partial digestion, with *S. aureus* V8 proteinase, of the overall heavy-chain band material cut off a preparative 15%-polyacrylamide slab gel (see the Materials and methods section). The results (Fig. 3) showed little differences in peptide pattern between digests of myosin heavy chains of foetal

Fig. 1. Electrophoretic analysis of myosin heavy chains of human developing and adult muscles

SDS/5% (w/v)-polyacrylamide-slab-gel electrophoresis of myosins was performed as described in the Materials and methods section. Key to muscle myosins: (i) foetal muscle; (ii) normal neonatal muscle; (iii) co-electrophoresis of (i)+(ii); (iv) anencephalic neonatal muscle; (v) immature neonatal muscle; (vi) co-electrophoresis of (i)+(iv); (vii) co-electrophoresis of (i)+(v); (viii) normal adult muscle (vastus lateralis). About 200 ng of protein were loaded per well. The slab was stained with silver nitrate as described by Merrill *et al.* (1981). Abbreviations used: HcF, adult fast myosin heavy chain; HCs, adult slow myosin heavy chain; HC_{emb.}, myosin heavy chain specific to foetal and neonatal muscle.

Fig. 2. Immunoenzymic staining of human myosin heavy chains with anti-(rabbit soleus myosin) antibody. Myosin heavy chains were resolved by slab-gel electrophoresis under conditions identical with those reported in Fig. 1. Half of the gel was stained with silver nitrate to reveal the protein bands (a), the other half of the gel was transferred on to a nitrocellulose sheet for immunoenzymic staining (b), as follows. The blot was incubated with chicken anti-(rabbit soleus myosin) affinity-purified antibody (10 µg of protein/ml) and alkaline phosphatase-conjugated anti-chicken IgG (see the Materials and methods section, Procedure B). Key to muscle myosins: (i) foetal muscle; (ii) anencephalic neonatal muscle; (iii) normal adult muscle. Abbreviations are as in Fig. 1.

Fig. 3. Peptide maps of myosin heavy chains

One-dimensional peptide mapping of myosin heavy chains was performed by a modification (Carraro *et al.*, 1981) of the procedure of Cleveland *et al.* (1977), by using heavy-chain bands cut out of preparative slab gels. The digestion was carried out with 25 µg of *S. aureus* V8 proteinase per slab gel. Key to lanes: (i) foetal muscle; (ii) normal neonatal muscle; (iii) anencephalic neonatal muscle; (iv) normal adult muscle. About 30–40 µg of protein were loaded per gel. Arrow indicates the *S. aureus* V8 proteinase.

and neonatal muscle and a distinct peptide pattern for adult myosin heavy chains, the latter being a composite of breakdown products of fast and slow heavy chains. In agreement with the electrophoretic evidence showing an increased amount of a slow-type heavy-chain component in the myosin of the anencephalic infant, electrophoretic analysis of the respective digest revealed the presence of several common peptides with adult myosin heavy chains.

Since electrophoretic evidence indicated that an identical major heavy-chain component is shared by foetal and normal neonatal myosin, we investigated the nature of this component by means of affinity-purified antibodies to human foetal myosin.

To be sure that the antibody was directed mainly towards antigenic determinants on the heavy chains, we used immunoenzymic staining techniques on blots of myosin/15%-polyacrylamide slab gels. It was verified that there was quantitative transfer both of the heavy and the light chains, and that, under these conditions, there was no visible staining in the light-chain region (Fig. 4a). When resolved by electrophoresis in SDS/5%-polyacrylamide gels, the two forms of adult heavy chains were only weakly stained by the antibody, as compared with the foetal-specific heavy chain

(Fig. 4b). As shown by immunoenzymic staining of blots of *S. aureus* V8 proteinase digests of the foetal-specific heavy chain with anti-(human foetal myosin) affinity-purified antibody, the antigenic determinants appeared to be distributed among several peptides, presumably derived from different domains of the heavy chains (Fig. 4c).

As shown in Fig. 5, the antibody was able to distinguish between the myosin of foetal and adult muscle when tested by e.l.i.s.a. techniques. On the other hand, no obvious antigenic differences could be demonstrated between foetal and neonatal myosin and, in the competitive assay, the concentration of antigen required for 50% inhibition of antibody binding to immobilized foetal myosin was found to be approximately the same (Fig. 5b). As expected, the extent of immunological cross-reactivity of anencephalic neonatal myosin with foetal myosin, was found to be decreased, by about one order of magnitude, as compared with normal neonatal myosin (Fig. 5b).

(b) Light-chain complement of human foetal and neonatal myosins

In view of the recent demonstration of a foetal-specific light chain in the myosin of human immature muscle (Strohman *et al.*, 1983; Cummins, 1983), we re-examined the light-chain pattern of foetal and neonatal myosins against that of adult myosin.

When analysed by one-dimensional electrophoresis in slightly overloaded slab gels (Fig. 6), both foetal and neonatal myosins gave rise to two main components in the light-chain region, representing the LC1F and LC2F light chains of adult myosin, in agreement with our earlier observations (Volpe *et al.*, 1981). Foetal myosin contained two additional light-chain components: a LC2S light chain and the previously described (Strohman *et al.*, 1983; Cummins, 1983) LC_{emb.}. Although neither of these two light chains appeared to be a normal component of muscle myosins of term and premature infants, the LC2S-light-chain band was found to be particularly prominent in muscle myosins from an anencephalic infant (Fig. 6). It was verified that all these differences between foetal and neonatal myosins were maintained after one purification cycle of the myosin preparations in pyrophosphate gels as described by Hoh (1978) and Fitzsimons & Hoh (1981a).

The results of Fig. 6 indicate an apparent M_r for human myosin LC_{emb.} of about 27000, which is higher than that of LC1F and at variance with the situation in the rat (Whalen *et al.*, 1978). It is also higher than that of the LC1Sa light chain (apparent M_r 26500), already shown to be a very minor component of human adult myosin (Fitzsimons & Hoh, 1981b). As shown by two-dimen-

Fig. 4. Immunoenzymic staining with anti-(human foetal myosin) antibody of foetal-specific myosin heavy chain and of its cleavage products after partial digestion with *S. aureus* V8 proteinase

(a) One-dimensional electrophoresis of foetal myosin was carried out in duplicate in 15%-polyacrylamide slab gels (about 40 μ g of protein per well). (i), Coomassie Blue staining of electrophoretic slab gel; (ii), immunostaining of blot of gel with affinity-purified anti-(human foetal myosin) antibody (10 μ g of protein per ml), as described in the Materials and methods section (Procedure A). The position of the myosin heavy-chain band, of contaminating actin and of myosin light chains are indicated. (b) One-dimensional electrophoresis of foetal myosin (i, iii) and of adult myosin (ii, iv) was carried out in 5%-polyacrylamide slab gels (about 200 ng of protein) under conditions identical with those reported in Fig. 1. Only the heavy-chain region is shown. (i, ii) Coomassie Blue-stained gels; (iii, iv) immunoblots obtained as in (a). Abbreviations are as in Fig. 1. (c) Immunoblot of *S. aureus* V8 proteinase digest of heavy-chain band cut out of a preparative slab gel of human foetal myosin as in (a). The digestion was carried out as described in Fig. 3.

sional gel electrophoresis (Fig. 7), human LC1Sa is contradistinguished by a very low isoelectric point, this also being lower than that of its rabbit slow-myosin homologue. These findings once more emphasize the existence of species-specific peculiarities of human myosin light chains (Volpe *et al.*, 1981).

(c) Antigenic and structural relationship between foetal-specific and adult myosin light chains

The antigenic and structural relationship between human myosin LC_{emb} and the LC1 components of adult slow skeletal muscle (Strohm *et al.*, 1983) and atrial muscle (Cummins, 1983) myosin is still controversial.

Immunoenzymic staining of the electrophoretically separated light chains of human foetal myosin with anti-(rabbit LC1F) antibody (Fig. 7) revealed that antibody staining is much weaker for the foetal-specific light chain, as compared with

LC1F, and qualitatively similar to that of LC2F, already shown to be weakly cross-reactive with anti-(rabbit LC1F) antibody by e.l.i.s.a. techniques (Biral *et al.*, 1982).

A structural heterogeneity between human foetal-specific light chain, LC1F, and the LC1S light chain is supported by the results obtained by peptide-map analysis. In these experiments, the foetal-specific light chain band, LC1F, and the overall LC1S material (predominantly LC1Sb) were cut out from preparative slab gels of myosin preparations from foetal, neonatal and adult muscle, and digested with *S. aureus* V8 proteinase. One-dimensional-peptide-map analysis (Fig. 8) by the method of Cleveland *et al.* (1977) showed an identical pattern for the LC1F component of human foetal, neonatal and adult myosin. This was similar to that of rabbit LC1F, as expected from the highly conserved structure of this light chain (Reeburgh Keller & Emerson, 1980; Rushbrook *et*

Fig. 5. Immunological cross-reactivities of the myosins of immature and adult muscles with anti-(human foetal myosin) antibody by e.l.i.s.a. techniques

(a) One step e.l.i.s.a. The immunoassay was carried out at antigen concentrations identical to those in Fig. 3 and at the concentrations of affinity-purified anti-(human foetal myosin) antibody indicated on the abscissa. (b) Two-step competitive e.l.i.s.a. The immunoassay was carried out as previously described (Biral *et al.*, 1982). Myosins, at the final concentrations indicated on the abscissa, were preincubated with anti-(human foetal myosin) antibody (5 μg of protein/ml). After incubating for 16 h, the second step was performed with microtitre wells coated with human foetal myosin (5 μg/ml). Key to muscle myosins: ●, foetal muscle; ○, normal neonatal muscle; □, immature neonatal muscle; ■, anencephalic newborn; Δ, normal adult muscle (pectoralis minor).

Fig. 6. SDS/polyacrylamide-gel electrophoresis of human muscle myosins

One-dimensional SDS/polyacrylamide-gel electrophoresis of muscle myosins was carried out in 15%-polyacrylamide slab gels as described by Laemmli (1970). Key to lanes: (a): (i) adult vastus lateralis myosin; (ii) adult vastus medialis myosin; (iii) foetal myosin; (b): (i) foetal myosin; (ii) normal neonatal myosin; (iii) anencephalic neonatal myosin; (iv) immature neonatal myosin. About 50 μg of protein (a) or 30 μg of protein (b) were loaded per well. The slab gels were stained with Coomassie Blue. The positions of the light chains of adult slow myosin (LC1Sa, LC1Sb, LC2S), fast myosin (LC1F, LC2F, LC3F) and of the foetal-specific light chain (LC_{emb}) are indicated.

Fig. 7. Two-dimensional electrophoresis of human muscle myosins and immunoenzymic-staining of foetal myosin light chains with anti-(rabbit LC1F) antibody

Two-dimensional electrophoresis of myosin samples was carried out as described in the Materials and methods section, on about 40 μ g of protein. The separated light chains on gels (a)–(c) were stained with Coomassie Blue. For immunoenzymic staining of foetal myosin light chains, a blot of the electrophoretic gel (d) was incubated with affinity-purified anti-(rabbit LC1F) antibody (10 μ g of protein/ml) and alkaline-phosphatase-conjugated anti-(chicken IgG), as reported in the Materials and methods section (Procedure A). Key to muscle myosins: (a) human normal adult muscle; (b) coelectrophoresis of human adult myosin (20 μ g of protein) and rabbit slow-muscle myosin (20 μ g of protein); (c) human foetal myosin (Coomassie Blue staining); (d) human foetal myosin (immunoenzymic staining). The spots corresponding to human fast-type (LC1F and LC2F) and slow-type (LC1Sa, LC1Sb, LC2S) light chains and to rabbit LC1S components (rLC1Sa, rLC1Sb), are labelled as indicated. Only the light-chain region is shown.

al., 1982). At variance with the situation in other mammalian species (e.g. the rabbit and the rat; results not shown), the peptide-map pattern of human LC1F appeared also to be qualitatively similar to that of its slow-type homologue (Fig. 8). On the other hand, the foetal-specific light chain exhibited a unique peptide pattern in all respects (Fig. 8).

Discussion

The results reported here, along with previous findings reported in the literature, outline certain common features as well as differences between human developing muscle and embryonic and neonatal muscle of other species.

The existence of an embryonic form of myosin light chain in human foetal muscle (Cummins, 1983; Strohmman *et al.*, 1983) is confirmed by our present results and appears to be a common feature

of myosin ontogenesis in mammalian, but not in avian, species (Whalen *et al.*, 1978; Gauthier *et al.*, 1982; see also Caplan *et al.*, 1983, for a review). In all species where it was demonstrated, its disappearance from myosin appears to be coincident with developmental periods at or around birth, as was also shown in the present study. Myosin LC_{emb.} has unique structural properties and, superimposed on these, species-specific features. Our results show that the peptide-map pattern of human LC_{emb.} is quite distinct from that of adult myosin LC1 components, thus disputing the suggested classification of this light chain as a slow type of light chain (Strohmman *et al.*, 1983). We also demonstrate that this light chain, unlike its rat homologue (Whalen *et al.*, 1978), has an isoelectric point identical with, but a greater molecular size than, that of LC1F. Because of these variations in structural details depending on the species, it may therefore be premature to exclude the possibility

Fig. 8. Peptide maps of myosin light chains

One-dimensional peptide mapping of myosin light chains was performed with *S. aureus* V8 proteinase as described in the Materials and methods section by the procedure of Cleveland *et al.* (1977) (a; 2 µg of proteinase/well) or a modification of it (Carraro *et al.*, 1981) (b; 25 µg of proteinase/slab gel) by using light-chain bands cut out of 15% polyacrylamide preparative slab gels. Key: (a): (i) overall LC1S band (mostly LC1Sb) from adult muscle myosin; (ii) LC1F from foetal muscle myosin; (iii) LC_{emb} from foetal muscle myosin. (b): (i) myosin LC1F from rabbit fast muscle; (ii) human foetal muscle; and (iii) from adult vastus lateralis. *S. aureus* V8 proteinase (*M_r* 30000), human LC1F (*M_r* 25000) and LC2F (*M_r* 18000) light chains were used as *M_r* markers.

that a hitherto-unidentified embryonic form of myosin light chain may be synthesized during early muscle ontogenesis in non-mammalian species.

A problem that has been at the origin of much controversy is the co-expression, in early developing muscle, of adult slow-type light chains (Stockdale *et al.*, 1981; Gauthier *et al.*, 1982; see also Caplan *et al.*, 1983). In human foetal muscle we also find a trace amount of LC2S, and the parallel observation of an adult slow electrophoretic form of heavy chain immunologically cross-reactive with the adult form suggests that a slow myosin isoenzyme is a normal component of early muscle myosin.

However, in spite of these analogies, interpretation of our findings is made difficult by the recent demonstration that embryonic slow myosin has a different heavy-chain peptide composition from that of the adult slow form (Benfield *et al.*, 1983). This also depends on the future differentiation of the muscles (Lyons *et al.*, 1983). Furthermore, our results indicate that the slow myosin component does not normally persist in neonatal muscle, except in muscle from an anencephalic infant, an observation that might suggest that, under these conditions at least, an altered pattern of discharge of spinal motoneurons due to the suppression of suprasegmental influences is the responsible factor. That would be contrary to the now-prevailing view (Benfield *et al.*, 1983; Lyons *et al.*, 1983) that non-neural intrinsic factors regulate the expression of myosin genes in early skeletal muscle. However,

our results do not necessarily imply that the nature of the slow myosin component is the same for foetal and pathological neonatal muscle.

With regard to transitions of myosin isoenzymes from foetal to neonatal life, our present results provide definitive evidence that, in leg muscle of full-term infants, only LC1F and LC2F are expressed, an observation similar to that in other species (Whalen *et al.*, 1981; Stockdale *et al.*, 1981; Gauthier *et al.*, 1982), and that the associated heavy chains are of a distinctive type from adult fast and slow myosin heavy chains. Nevertheless, there is no evidence to indicate that, in humans, the 'neonatal' form of myosin heavy chains is structurally and antigenically different from the predominant foetal form. That is apparently in contradiction with knowledge of the sequential appearance of an embryonic and a neonatal form of myosin heavy chain during muscle development, as originally described by Whalen *et al.* (1981) in the rat. The chronological sequence, in absolute times, of these transitions, was, however, shown to vary considerably according to the species (Lowey *et al.*, 1983), as well as between future fast and slow muscles of the rat (Lyons *et al.*, 1983).

Owing to the inherent limitations of the analytical techniques used, we cannot entirely exclude the existence of subtle structural differences between human foetal and neonatal heavy chains. However, an alternative explanation is that, in the human species, which spends a considerable time

to reach full maturity, an embryonic/foetal form of myosin heavy chains may persist in muscle until birth and be substituted by a 'neonatal' form at later periods of postnatal life.

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