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Three-Dimensional Structure of the Perimysium in Sternocleidomastoid Muscle

By

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Summary: The morphology of the sternocleidomastoid muscle (SCM) of human fetuses, ranged from 12 to 32 weeks gestation, was investigated by a light microscopy and a scanning electron microscopy. The collagenous fibers of the perimysium of the SCM formed complex structures from 24 weeks gestation by contrast to fibers of the endomysium of the SCM. The cross-sectional area (CSA) of the bundle of muscle fibers and the CSA of the individual muscle fibers of the SCM increased during development from 12 to 32 weeks gestation, in a process linked to the development of the perimysium. Therefore the perimysium affects and controls to the muscle fiber of the developed SCM and acts to resisting stretch forces in the movements. The changes in the arrangement and development of the collagenous fibers in the perimysium may be correlated to with these of the muscle fibers.

Vries *et al.* (1982) reported that rotation of the head movement occurs from 9 week to 13 weeks gestation and is often associated with retroflexions of the head. Hooker (1952) and Birnholz *et al.* (1978) also reported that head movement occurs at early stage in the fetus. The differentiation of fiber types in developing human skeletal muscle is found from an early stage (ca. 10–21 weeks gestations; Dubowitz, 1963, 1965, 1966; Colling-Saltin, 1978). The myotube fibers are clearly visibly at an early, stage at least before 12 weeks gestation, and formation of muscle fibers can be seen 20–24 weeks gestations. These reports indicate that the organization of muscle occurs at early stages during formation of and changes in muscle fibers and affects development.

In general, collagenous fibrils of connective tissues can be classified into two types: large, straight parallel fibrils (Fawcett, 1986); and helically arranged, wavy fibrils that form sinuous bundles or three-dimensional sheaths (Rizk, 1980). The connective tissues are composed of type I, type III, and type V which indicate intermixed fibrils by chemical and immunohistochemical methods (Light and Champion, 1984; Mayne and Sanderson, 1985). It is difficult to determine the compositions of fiber types, and to determine changes in size of collagen fibers and patterns in different regions of the muscle during development. Such information is important if we are to understand the function of the connective tissues of the muscle fibers. The framework with its collagen fibrils that surrounds each muscle fiber is also involved in resisting stretching forces during movement (Schmalbruch, 1974). The development of the connective tissue of the muscle may be involved in the formation and development of the muscle fiber itself. There are many factors that affect the development of muscle, for example, the extracellular matrix components (ECM) are related to the pattern of muscle fibers (Chevallier *et al.*, 1977; Kieny and Chevallier, 1980). Collagen is required by organisms when they become multicellular and develop tissues and organs (Schmidt and Alder, 1984; Reh and Nagy, 1987; Park and Hollenberg, 1989). Transforming growth factor beta 1 is implicated in the formation of patterns of muscle fibers (McLennan, 1993) in addition to temporal and positional factors (McLennan, 1983; Narusawa *et al.*, 1987). The organized layers, suggested by the earlier studies, namely the connective tissue of the muscle, are the most important elements to in the development of muscle fibers.

In the present study, we examined the morphology

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of collagenous fibers and the relationship between the muscle fibers and the connective tissue in human fetuses, which ranged from 12 to 32 weeks gestation, by light microscopy and scanning electron microscopy.

Materials and Methods

Twelve fetuses at autopsy were selected from donations at Nippon Dental University for morphological analysis of the sternocleidomastoid (SCM). The approximate fetal age was assigned by reference to Streeter's Tables (Streeter, 1920, 1942, 1948, 1951). The specimens were fixed for 30 min in a solution of 10% paraformalin. After specimens had been washed in running water, the SCM was dehydrated in absolute ethyl alcohol and then embedded in paraffin. Serial cross sections were made at a thickness of about 3 μm on a rotary microtome. Picro-sirus red staining was used in order to determinate between the epimysium and perimysium (Sweet *et al.*, 1964). Sections were observed under a light microscope (Vanox-S; Olympas Co., Tokyo). The cross-sectional area (CSA) of muscle (mm^2) and of individual muscle fibers (μm^2) in each muscle section were measured into an image analyzer (Pias LA-500; Pias Co., Osaka) linked to a microcomputer (PC-9801 VX; NEC, Tokyo) (Fig. 1). Fifty areas were selected at random for analysis of muscle fibers.

Four sections were selected from serial sections that had been fixed in 0.2 M glutaraldehyde and post-fixed in 1% osmic acid. Sections were washed in 2% cacodylate buffer (pH 7.2) after dewaxing, and then etched with 8 N-HCl for 3 sec. at room temperature (modified version of the method of Desaki and Uehara, 1981) and finally dried by a t-butyl alcohol freeze-drying method (Inoue and Osatake, 1988). They were coated with a 1.5-nm layer of gold-palladium and observed under a scanning electron microscope (S-4000; Hitachi Co., Tokyo; 5-10 kv).

Results

Microscopic observations of the sternocleidomastoid muscle

At 12 weeks gestation, myotube cells were found in cross-sections of the sternocleidomastoid muscle (SCM). Irregularly arranged, fine shows of connective tissues were seen around these muscle fibers (Fig. 2a). The perimysium, composed of thin shown of connective tissues reacted very weakly with picro-sirus red, but there were no large bundles of collagen fibers. From 16 weeks gestation, collagenous bundles were found in the epimysium (Fig. 2b). The perimysium was composed of irregularly arranged collagenous bundles and reacted strongly with picro-sirus red from 20 weeks (Fig. 2c). The perimysium was composed of thick and thin collagen fibers. At 28–32 weeks, numerous collagen fibers were arranged in irregular patterns and formed a network between each muscle fiber (Figs. 2d-f).

Dimensions of the sternocleidomastoid muscle

Weights of muscle and the cross-sectional areas (CSA) of muscle and individual muscle fibers are shown in Fig. 3. The weight and the CSA of the muscle in the SCM increased rapidly from 2 weeks gestation. However, the weight and the CSA of the SCM had already begun to increase gradually from 12 to 20 weeks gestation. The CSA of individual muscle fibers of the SCM increased gradually throughout development and increased rapidly from 28 weeks gestation.

Scanning electron microscopy

At 12 weeks gestation, the muscle fibers contained numerous myofibrils which formed a hollow core (Fig. 4a). These myofibrils were surrounded by thin reticular fibers. The few very fine irregularly arranged fibers (about 1 nm in diameter) were found around muscle fibers, and they run from muscle fiber to the other muscle fibers. The thin perimysium was

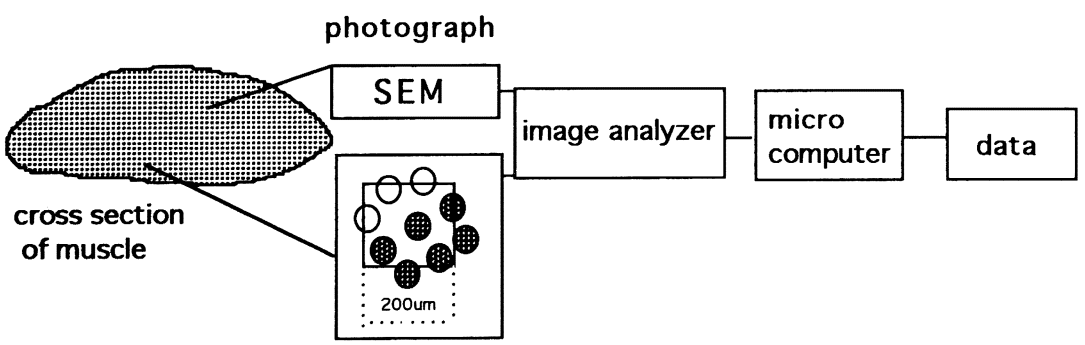

Fig. 1. Schematic representation of the analytical method. Data (dimensions of muscle fibers) were obtained by use of a light microscope linked to an image analyzer and a computer system. Small regions [white-outlined square: cross-sectional area (40000 μm^2) of muscle fiber] were prepared for analysis.

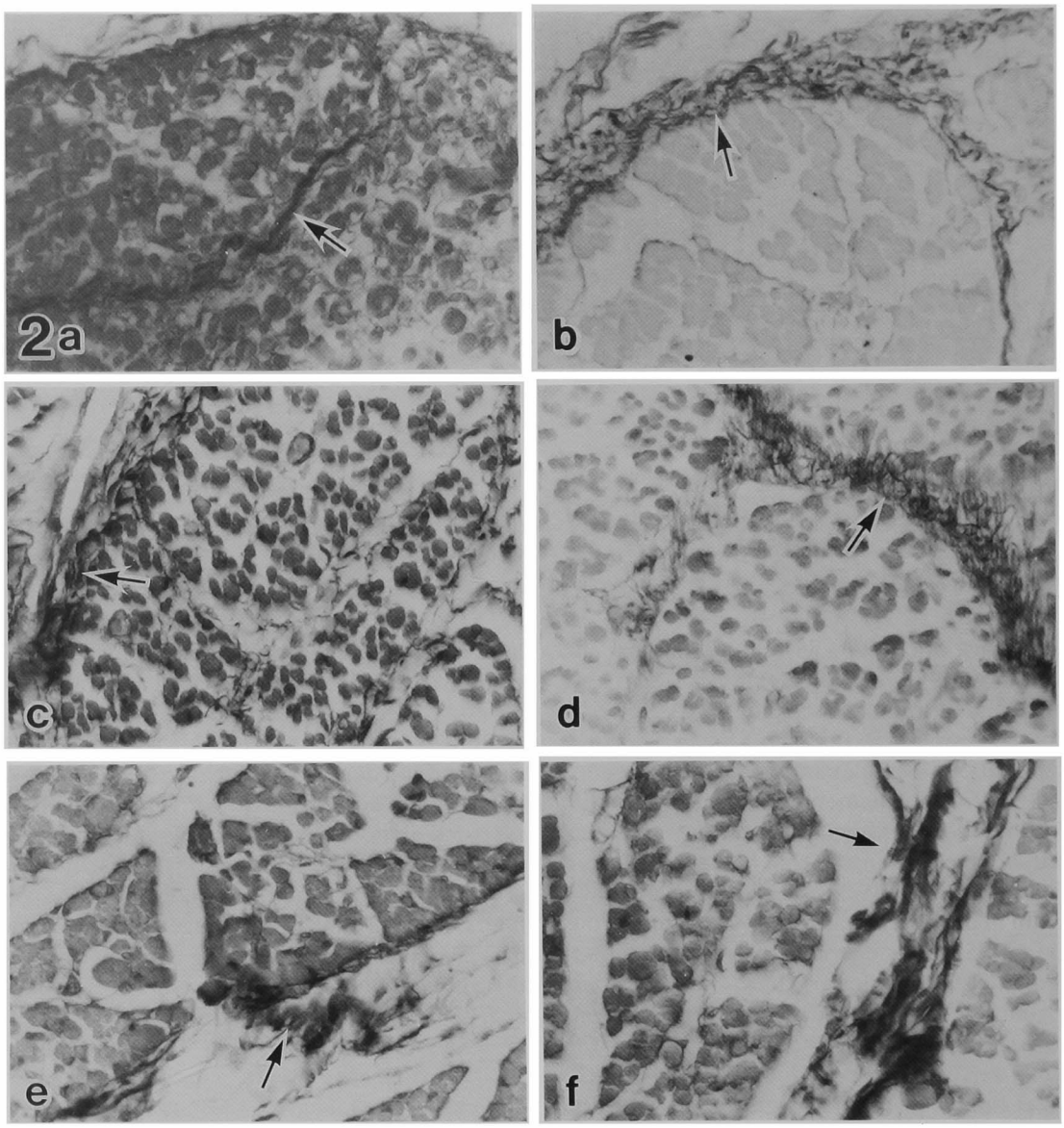

Fig. 2. Light micrographs of cross sections of muscle fibers in the human sternocleidomastoid muscle (SCM) stained with picrosirus red. **a.** At 12 weeks gestation, the perimysium (arrow) is composed of complex structures near the muscle fibers. **b.** At 16 weeks gestation, the perimysium (arrow) is composed of irregularly arranged collagenous fibers. **c.** At 20 weeks gestation, the perimysium forms a complex network structure (arrow), with fine fibers between each muscle fiber. **d.** At 24 weeks gestation, the perimysium is composed of large bundles (arrow) arranged irregularly. **e.** At 28 weeks gestation, a very large perimysium is composed a complex network structure. **f.** At 32 weeks gestation, a few large bundles (arrow) form the perimysium and are arranged irregularly (Bar = 50 μm).

composed of an irregular arrangement of thick (40–60 nm in diameter) and complex thin fibers (about 20 nm in diameter) (Fig. 4a). By 16 weeks gestation, the number of collagenous fibers had gradually increased and they formed networks which connected with muscle fibers. Numerous large and small fibers were found in the perimysium (Fig. 4b). In the epimysium, irregular, thick collagen fibers and thin reticular fibers were found and seen to form networks (Fig. 4b). At 20 weeks, in the perimysium, large and small collagen fibers formed thick bundles (about 4.5 μm in diameter) (Fig. 4c). The perimysium was composed of a complex network of numerous thick and thin fibers. Some of small bundles were composed of thick and thin collagen fibers, and these bundles were arranged regularly oriented parallel to the longitudinal axis of the muscle fiber. The numerous fine fibers (20–40 nm in diameter) formed fine networks

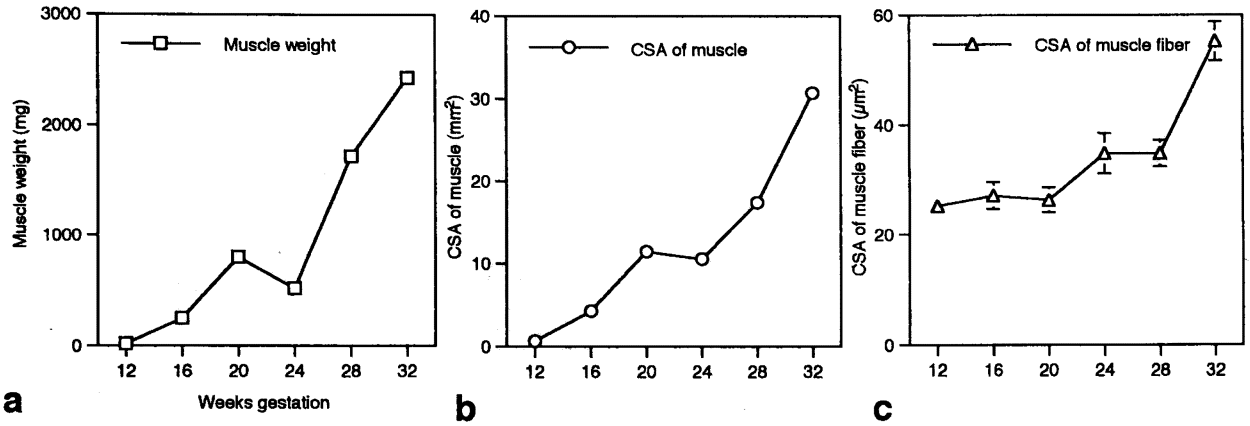

Fig. 3. Dimension of the sternocleidomastoid muscle
a. Weight of the sternocleidomastoid muscle from 12 to 32 weeks gestation
b. Cross section of the sternocleidomastoid muscle from 12 to 32 weeks gestation
c. Cross section of muscle fiber of the sternocleidomastoid muscle from 12 to 32 weeks gestation

around the thick fibers (60–80 nm in diameter) and bundles (about 0.6 μm in diameter) (Fig. 4c). These layers were linked to the muscle fibers. At 24 weeks gestation, the perimysium (about 5.5 μm in diameter) was composed of a complex network of collagenous fibers (60–100 nm in diameter) and numerous fine networks of reticular fibers. The network of thick fibers and the fine network of thin fibers were linked each others (Fig. 4d). At 28 weeks, the perimysium (about 8 μm in diameter) was composed of numerous thin (about 0.5 μm in diameter) and thick (about 4 μm in diameter) bundles of thick collagen fibers (80–100 nm in diameter), and these bundles were arranged regularly and oriented in parallel to the longitudinal axis of the muscle (Fig. 4e). Each muscle bundle was linked to thick collagen fibers and fine reticular fibers which formed complex networks (Fig. 4e). At 32 weeks, numerous thin fibers (20–40 nm in diameter) and thick fibers (60–100 nm in diameter) formed a complex network which linked the muscle fibers (Fig. 4f). Each muscle bundle was linked to thick and think collagen fibers. In the perimysium (about 18 μm in diameter), a large number of thick collagenous bundles (about 3 μm in diameter) was arranged irregularly. Some of the connective tissue near the muscle fibers formed a complex network composed of thick and thin fibers (Fig. 4f).

Discussion

The sternocleidomastoid muscle (SCM) is a 'rotator' type of muscle and functions in turning movements around a longitudinal axis. Strong stress is exhibited when it acts in the flexion, rotation and lateral flexion of the head. In mice, the SCM has a complex collagenous matrix which act as a framework for the rotating structures (Nagel, 1935). The SCM of human fetuses has a complex collagenous epimysium and the density of fibers increases during development. In our SEM study, Ohtani *et al.* (1988) reported that the endomysium of the dog lingual muscle is composed of two types of fibers which are separated by different arrangements of collagen fibers. Helically arranged collagen fibrils surrounding muscle fibers act to resisting stretch forces along the muscle fibers (Schmalbrauch, 1974). Bundles of collagen fibrils allow freedom of motion individual muscle fibers (Fawcett, 1986). It appears, therefore, that the connective tissues in each muscle play different roles in movement. In the human fetus, individual muscle bundles were separated from each others by connective tissues (perimysium): reticular and collagen fibers. The connective tissues of the SCM increased in density. In particular, a large number of collagen fibers and complex networks were found in the SCM during development. The morphological features and composition of reticular and collagen fibers may be directly related to the functional properties of each muscle. The endomysium composed of curvilinear collagen fibrils in an isotropic array has been found in various animals (breast muscle of domestic fowl; Bennett and Porter, 1953; tibialis anticus muscle of frog; Mauro and Adamus, 1961; biceps femoris of cat, Trotter and Purslow, 1992), while other studies have shown as two types of the collagen fiber (sartorius muscle; Schmalbruch, 1974; ventral abdominal wall muscle of albino rat; Risk, 1980; human cardiac muscle; Ohtani *et al.*, 1988). Fawcett (1986) reported that collagen fibers play a role in the freedom of

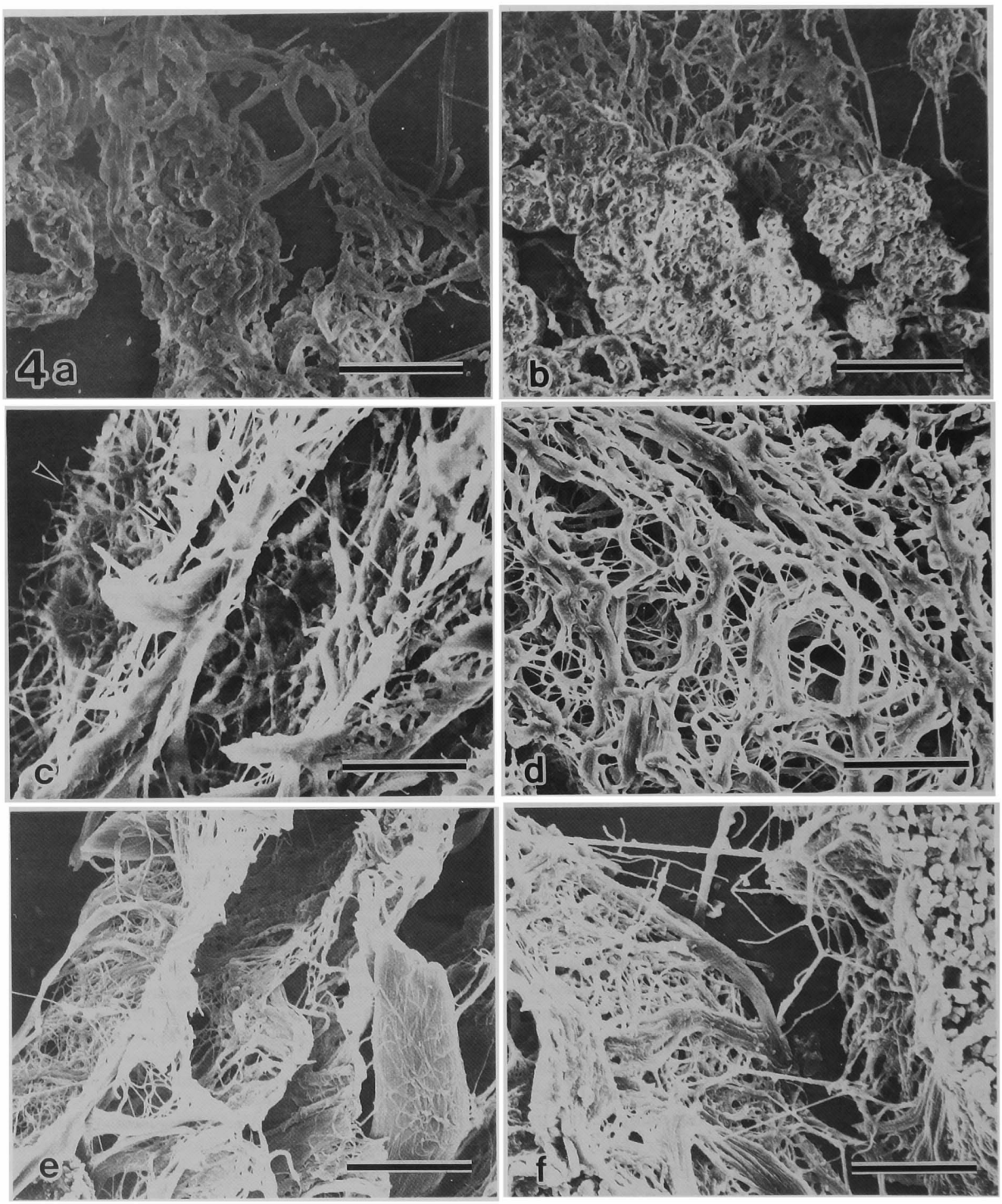

Fig. 4. A scanning electron micrograph of the cross section of muscle fibers in the human sternocleidomastoid muscle.
a. At the 12 week gestations, the few number of very fine fibers are found around the muscle fiber cells, and they run from the muscle fiber to the other muscle fibers (Bar = 5 μm). **b.** At 16 weeks gestation, numerous thick and thin fibers are found in the perimysium. Complex thick collagen fibers and small reticular fibers are seen as networks (Bar = 7.5 μm). **c.** At 20 weeks gestation, thick bundles composed of complex network of thick and thin fibers form the perimysium. Numerous fine fibers formed networks around large fibers and the perimysium composed of irregular arrangement of large (arrow) and complex small (arrowhead) fibers (Bar = 2.5 μm). **d.** At 24 weeks gestation, the perimysium is composed of complex collagenous networks and fine networks of reticular fibers. The small fine fibrous networks are linked to one another (Bar = 3.3 μm). **e.** At 28 weeks gestation, the perimysium is composed of numerous bundles of thick collagen fibers which are linked via thick collagen fibers. Fine reticular fibers are form complex networks (Bar = 2.2 μm). **f.** At 32 weeks gestation, numerous fine fibers and thick fibers form complex networks. Large numbers of thick collagenous bundles are arranged irregularly. Some of the connective tissue near the muscle fibers forms a complex network (Bar = 3.8 μm).

motion of individual muscle fibers during movement. Schmalbruch (1985) suggested that the collagen fibers that surround muscle fibers protect the muscle fiber during movements. The morphological features reflect functional differentiation within the muscle. The endomysium is composed of helically arranged and longitudinal bundles of collagen fibrils (Plenk, 1927, 1934; Ushiki and Ide, 1986; Ohtani *et al.*, 1988), which are stained in silver-impregnated preparations (Laidlaw, 1930; Nageotte, 1932). In our observations, scanning electron microscopy revealed that these collagen fibers were composed of thick and thin fibrils which increased in number and formed a complex network from 16 weeks gestation. Collagen fibers control formation of myotube cells. Gulati *et al.* (1982) reported that the appearance of fibronectin is associated with the formation of myotubes. Fibronectin is located in pericellular regions of the myotubes and is absent from the sarcoplasm (Linder *et al.*, 1978; Stenman and Vaheri, 1978). The manubrium becomes visible from the muscle cell by a trans differentiation with collagenous enzymes (Schmidt, 1984). The connective tissues contain myogenic regulatory factors (Davis *et al.*, 1987). Ontell (1982) reported that the formation of the perimysium affects the development of the fascicles of muscles (Ignotz and Massague, 1986; Varga *et al.*, 1987; Ishikawa *et al.*, 1990). McLennan (1993) suggested that the TGF-β1 is affect to the fibroblasts and other connective tissue. The TGF-β1 affect also to the development of myoblasts. Colling-Saltin reported (1978) that large type I muscle fibers occurred at 20 weeks gestation. A difference in levels of growth factor may be involved in the connective tissue around the differentiation of types of muscle fiber at each embryonic stages.

The connective tissue network of the epimysium, perimysium and endomysium in the skeletal muscle is composed of type I and type III collagen (Mayne and Sanderson, 1985). The distribution of the various collagen fibers differs in the muscle (Light and Champion, 1984). The morphological features of the extracellular matrix network have been implicated in heart function (Borg *et al.*, 1983; Weber *et al.*, 1987; Borg and Terracio, 1990; Carver *et al.*, 1993). In particular, the level of type III collagen in the neonatal heart increases in contrast to that of type I collagen (Carver *et al.*, 1993). The distribution of the different types of collagen suggests differences in the functional properties of other skeletal muscles. In the SCM of the human fetus, the thick collagen fibrils increased in number during development of the fetus. The complex network of collagen fibers of the perimysium in the SCM is implicated the freedom of movement of the muscle fibers and allows allow the specialized 'rotator' actions of the muscle that is associated with a considerable stress.

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