The Endomysium of Human Embryonic Hyoid Muscle during Development

Iwao SATO, Masataka SUNOHARA, Toshiya FUJITA and Toru SATO

Department of Anatomy, School of Dentistry at Tokyo, Nippon Dental University, Tokyo 102-8159 Japan

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Summary: A computer analysis was performed on the endomysium of the hyoid muscle in human fetuses, which ranged in gestational week from 12 to 32 weeks. During development of the human hyoid muscle a level of intramuscular connective tissue as reticular fibers displays a specific change in morphology. We examined quantities and distributions of endomysium in human hyoid muscles from 12 to 32 weeks. The endomysium of the hyoid muscles formed complex structures, and volume rates increased from 20 weeks gestation. On the other hand, the cross sectional area (CSA) of muscle fibers had almost the same profile. The reticular fibers increased during development from 16 to 28 weeks gestation.

Collagenous fibrils can be roughly classified into two types, straight parallel fibrils (Fawcett, 1986), and helically arranged, wavy fibrils. The collagenous fibrils form sinuous bundles and three-dimensional sheaths (Rizk, 1980). The organized layers of a muscle are composed of the epimysium, perimysium and endomysium. Endomysium, which separates from each muscle fiber and covers the surface muscle fiber, are called ensheaths (Walls, 1960). The connective tissue networks of these organizations in the skeletal muscle contained extracellular matrices with growth factors (Mayne and Sanderson, 1985). Sato and Shimada (1997) reported that the fibrile collagen of extracellular matrix affects development of masticatory muscles. The extracellular matrix components are related to patterns of muscle fibers (Chevallier *et al.*, 1977; Kieny and Chevallier, 1980). Transforming growth factor beta 1 is implicated in formation of patterns of muscle fibers (McLennan, 1993) in addition to temporal and positional factors (McLennan, 1983; Narusawa *et al.*, 1987).

This framework with its collagen fibrils that surround each muscle fiber is involved in resisting stretching forces during a movement (Schmalbruch, 1974). 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 (1978) also reported that the head movement occurs at an early stage of the fetus. The opening of the jaw occurs from 8.5 weeks (Humphrey, 1968) and sucking and swallowing with drinking amniotic

fluid is displayed from 12.5 weeks (Vries *et al.*, 1982). Nishimura *et al.* (1996) found intramuscular connective tissue of bovine semitendinous muscle decreased during development. Therefore, the morphology of the connective tissue as an endomysium is an important element in function and development of the human hyoid muscles.

In the present study, we examined the quantity of endomysium and the muscle fibers of the hyoid muscles during development in human fetuses, which ranged from 12 to 32 weeks gestation.

Materials and Methods

Twelve fetuses were processed at autopsy for the immunocytochemical analysis for the morphological analysis of the hyoid muscles (superior hyoid: anterior belly of digastric, posterior belly of digastric, mylohyoid, stylohyoid and geniohyoid; inferior hyoid: superior belly of omohyoid, inferior belly of omohyoid, sternohyoid, thyrohyoid and sternothyroid). The fetuses were obtained from legally approved, induced abortions and spontaneous abortions. After abortion, such investigations are part of the legally required autopsy. Twelve fetuses at autopsy were selected from donations at Nippon Dental University. The approximate fetal age was assigned by reference to Streeter's Tables (Streeter, 1920, 1942, 1948, 1951). These specimens were fixed for a 30 min in a solution of 10% paraformalin. After specimens had been washed in

running water, the hyoid muscles were dehydrated in absolute ethyl alcohol and then embedded in paraffin. Serial frontal sections were made at a thickness of about 3 μm on a rotary microtome. Reticular fiber was demonstrated in the intramuscular connective tissue of hyoid muscles using a silver impregnation technique (Sano, 1954) in order to differentiate between the epimysium and perimysium. Sections of muscle fibers were observed under a light microscope (Vanox-S; Olympus Co., Tokyo). The cross sectional area (CSA) of individual muscle fiber (μm^2) in each section were measured with an image analyzer (Pias LA-500, Pias Co., Osaka, Japan) linked to a microcomputer (PC-9801 VX, NEC, Tokyo, Japan). Ten areas were selected at random on the CSA of muscle fibers. The measurement areas for the ratio of reticular fiber per 10,000 μm^2 were selected from each section and measured with 400 magnification by a light microscope. The densitometric profiles of the reticular fiber with silver staining were determined with a color image analyzer (Image Hyper II, Interquest Co., Osaka, Japan) linked to a microcomputer (Dion, Tokyo, Japan; Fig. 1).

Results

Microscopic observations of the hyoid muscles

At 12 weeks gestation, large myotube cells were found in the cross section of the hyoid muscles. Reticular fibers (endomysium) with silver staining were found around these large muscle fibers (Fig. 2a). The endomysium showed a honeycomb-like structure surrounding the muscle fibers and the cross section of muscle fiber increased during development. From 16 weeks gestation, collagenous bundles of the perimysium were found around these small muscle fibers (Fig. 2b). The endomysium was made up of irregularly arranged fibers which reacted strongly with silver staining from 20 weeks (Fig. 2c). The fine reticular fibers were found around muscle fibers which has loose contact with the perimysium fiber bundles. At 28 and 32 weeks, numerous reticular fibers were arranged in irregular patterns and formed a network around muscle fiber (Figs. 2d-f).

Analysis of muscle fiber and reticular fiber with a computer system

The ratios of the reticular fiber (RF) in each section are shown in Fig. 3a,b. In the earlier stage (12 weeks gestation), the rates of RF with silver staining are almost 15% in anterior belly of digastric (AD), the stylohyoid (SH), the thyrohyoid (TH), the sternothyroid (ST). The rates of the RF

in other hyoid muscles are 10% below. The percentages of the RF increased gradually in the AD, the posterior belly of digastric (PD), the geniohyoid (GH), the inferior belly of omohyoid (IO), and the sternohyoid (STH) during development. The percentages of the RF are almost constant in the SH, the ST and the TH from 12 to 32 weeks. Although the percentage of the RF increased gradually from 24 weeks, it is constant low, ranging from 12 to 24 weeks in the superior belly of omohyoid (SO). The percentage of the RF increased strikingly from 12 to 20 weeks, and decreased at 24 weeks in the mylohyoid (MH).

The CSA of muscle fiber in hyoid muscle

In the earlier stage (12 weeks gestation), the CSA of muscle fiber is large in the inferior hyoid muscles as compared with that of the superior hyoid muscles. From 24 weeks gestation, the CSA of muscle fiber slightly increased. In contrast, the CSA of muscle fiber in the GH was still low and was constant, ranging from 12 to 32 weeks. From 12 to 24 weeks the CSA of muscle fiber was still low and increased gradually from 28 weeks in the AD, the MH, and the SH. At the 12 weeks the CSA of muscle fiber was large, and gradually decreased from 16 to 20 weeks, and finally increased at 28 and 32 weeks in the SO, the IO, and the TH. The CSA of muscle fiber gradually decreased from 12 to 20 weeks and was constant low in the PD and the STH. The ST maintained the same value at 12, 28, and 32 weeks, and the value was still lower at 16 and 24 weeks.

Discussion

The muscle fiber during development

In the early stage, the myotube fibers are clearly visible and the formation of muscle fibers can be seen at 20 and 24 weeks gestation. The myotube fiber is found before 12 weeks gestation (Dubowitz, 1963, 1965, 1966). The myoblasts are found round these myotube. These structures reflect the large size of the CSA of muscle fiber at early stage. Comparing superior with inferior hyoid muscles, the CSA of muscle fiber in inferior hyoid muscle, the SO, the IO, and the STH are large at 12 weeks. The muscle fiber of the inferior hyoid muscles can be undeveloped compared with superior hyoid muscles in these embryonic stages. In general the CSA of muscle fiber increased during development, and is only found in the SO, the IO and the TH from 16 weeks. Most hyoid muscles displayed various levels of the CSA in muscle fiber.

Function in hyoid muscle during development

Ohtani *et al.* (1988) reported that the endomysium of the dog lingual muscle was composed of two types of fibers which were separated by different arrangements of collagen fibers. Helically arranged collagen fibrils surrounding muscle fibers act to resist stretch forces along the muscle fibers (Schmalbrauch, 1974). Bundles of collagen fibrils allow freedom of motion of individual muscle fibers (Fawcett, 1986). Therefore, collagen fibrils in each muscle play different roles in movement. In particular, the ensheaths of the endomysium are relatively extensible in longitudinal directions. The mechanical strength of the endomysium of hyoid muscles increased during development when covered by fine complex fibers of the endomysium. Therefore, the endomysial ensheaths displayed provided reinforcement for their movements. In particular, a large number of collagen fibers and complex networks were found at postal stage during development. Fawcett (1986) reported that collagen fibers play a role in the freedom of motion of individual muscle fibers during movement. 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).

Development of reticular fiber

The two types of fibers are separated by different arrangements of collagen fibers in the endomysium (Ohtani *et al.*, 1988). Sato and Shimada (1997) found collagen fibers changed in volume and form at each stage in the development of postnatal rat masticatory muscle. In the first stage thin fibers formed irregularly arranged networks; in the second stage fibers increased in number. In the third stage, collagen bundles formed. In the fourth stage, these bundles increased in number, and in the fifth stage bundles became the thickest in development of the muscle fibers. In our results, although reticular fiber increased gradually, the CSA of muscle fiber did not show same profiles. The percentage of reticular fiber was high in value, in contrast with the low value of the CSA of muscle fiber at 20 weeks in inferior hyoid muscle; the IO, the STH, and the ST and the superior muscle; the MH. Purslow (1994) demonstrated reorientation of collagen fibrils in the endomysium with changing muscle length. During development the intramuscular connective tissue changed the form and the contents in each hyoid muscle associated with increasingly dense reticular fiber. Moreover, the specific profiles are associated with extracellular matrices. The connective tissue network of the epimysium, perimysium and endo-

mysium in the skeletal muscle is mainly composed of collagenous protein such as collagen types I and III (Mayne and Sanderson, 1985). Robinson *et al.* (1988) also reported that endomysium contained collagen types I and III in cardiac muscle. Other extracellular matrices affect cell morphology and the cytoskeletal organization of actin microfilaments (Kreis and Vale, 1993). Therefore, the morphology and the migration of the reticular fiber at each stage reflect the intensity of immunoreactions of proteins.

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Explanation of Figures

Plate I

Fig. 1. Densitometric profile of the endomysium of the cross sections of muscle fibers in the human anterior belly of digastric with a silver impregnation technique determined with a color image analyzer linked to a microcomputer. a, Color photograph with endomysium (black). b, Image obtained determined by computer analysis (bar = 100 μ m).

Plate II

Fig. 2. Light micrographs of cross sections of muscle fibers in the human anterior belly of digastric with a silver impregnation technique. a, At 12 weeks gestation, the endomysium is composed of complex structures near the muscle fibers. b, At 16 weeks gestation, the endomysium is composed of irregularly arranged fibers. c, At 20 weeks gestation, the endomysium forms a complex network structure, with fine fibers between each muscle fiber. d, At 24 weeks gestation, the endomysium is composed of large bundles arranged. e, At 28 weeks gestation, the endomysium is composed a complex network structure. f, At 32 weeks gestation, the endomysium is arranged regularly (Bar = 100 μ m).

Plate III

Fig. 3.a. Changes of percentage of reticular fiber (RF) in the intramuscular connective tissue using a silver staining and the cross sectional area of muscle fiber in the superior hyoid muscles. a, anterior belly of digastric; b, posterior belly of digastric; c, mylohyoid; d, stylohyoid; e, geniohyoid

Fig. 3.b. Changes of percentage of reticular fiber (RF) in the intramuscular connective tissue using a silver staining and the cross sectional area of muscle fiber in the inferior hyoid muscles. a, superior belly of omohyoid; b, inferior belly of omohyoid; c, sternohyoid; d, thyrohyoid; e, sternothyroid