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Cleft Palate Craniofac J. Author manuscript; available in PMC 2009 September 29.

Published in final edited form as:
Cleft Palate Craniofac J. 2008 March ; 45(2): 113–120. doi:10.1597/06-171.1.

Effect of Cleft Palate Repair on the Susceptibility to Contraction-Induced Injury of Single Permeabilized Muscle Fibers From Congenitally-Clefted Goat Palates

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Abstract

Objective—Despite cleft palate repair, velopharyngeal competence is not achieved in ~15% of patients, often necessitating secondary surgical correction. Velopharyngeal competence postrepair may require the conversion of levator veli palatini muscle fibers from injury-susceptible type 2 fibers to injury-resistant type 1 fibers. As an initial step to determining the validity of this theory, we tested the hypothesis that, in most cases, repair induces the transformation to type 1 fibers, thus diminishing susceptibility to injury.

Interventions—Single permeabilized levator veli palatini muscle fibers were obtained from normal palates and nonrepaired congenitally-clefted palates of young (2 months old) and adult (14 to 15 months old) goats and from repaired palates of adult goats (8 months old). Repair was done at 2 months of age using a modified von Langenbeck technique.

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Presented, in part, at the 63rd American Cleft Palate–Craniofacial Association, Vancouver, British Columbia, Canada, April 2006; the 51st Annual Meeting of the Plastic Surgery Research Council; and the Experimental Biology 2006 Annual Meeting.

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Main Outcome Measures—Fiber type was determined by contractile properties and susceptibility to injury was assessed by force deficit, the decrease in maximum force following a lengthening contraction protocol expressed as a percentage of initial force.

Results—For normal palates and cleft palates of young goats, the majority of the fibers were type 2 with force deficits of ~40%. Following repair, 80% of the fibers were type 1 with force deficits of 20% ± 2%; these deficits were 45% of those for nonrepaired cleft palates of adult goats ($p < .0001$).

Conclusion—The decrease in the percentage of type 2 fibers and susceptibility to injury may be important for the development of a functional levator veli palatini muscle postrepair.

Keywords

levator veli palatini; velopharyngeal incompetence

Orofacial cleft anomalies are the most prevalent birth defects in the United States (Centers for Disease Control and Prevention [CDC], 2006). Worldwide, congenital cleft palates occur at the rate of 7 per 10,000 live births (Elahi et al., 2004; Forrester and Merz, 2004; CDC, 2006). Following surgical repair, 10% to 20% of patients are still not able to achieve velopharyngeal closure (Marrinan et al., 1998), resulting in abnormal speech and other functional deficits that often necessitate secondary surgical correction. This outcome is typically a consequence of either inadequate palatal length or insufficient movement of the pharyngeal walls or soft palate. Soft palatal movement is dependent on the proper functioning of soft palate musculature (Kogo et al., 1996; Marrinan et al., 1998; Berry et al., 1999). Levator veli palatini (LVP; abbreviations listed in Table 1) muscle fibers from normal palates are aligned transversely across the posterior soft palate and when activated during velopharyngeal closure, retrodisplace and elevate the palate (Kogo et al., 1996; Marrinan et al., 1998; Berry et al., 1999). Impairment of LVP muscles of patients with cleft palates may contribute to the incidence of velopharyngeal dysfunction postrepair.

The functionality of the LVP muscle is dependent on the fiber-type composition (Hanes et al., 2006; Rader et al., 2006). For normal palates of adult humans (Stal and Lindman, 2000; Lindman et al., 2001) and goats (Hanes et al., 2006; Rader et al., 2006), LVP muscles consist primarily of slow oxidative (type 1) fibers. In contrast, the LVP muscles in nonrepaired cleft palates are oriented in a longitudinal direction, abnormally insert on the posterior hard palate, and at least for adult goats, are predominantly fast glycolytic or fast oxidative fibers, referred to collectively as type 2 fibers (Hanes et al., 2006; Rader et al., 2006). The fiber-type composition of skeletal muscle is affected by extrinsic factors such as the frequency of activation and loading characteristics (Rubinstein and Kelly, 1981; Hughes and Blau, 1992). The implication is that the misalignment of the muscle fibers in cleft palates results in abnormal loading of the fibers and subsequent maladaptive changes in the LVP, resulting in a higher percentage of type 2 fibers (Hanes et al., 2006; Rader et al., 2006).

Contraction-induced injury occurs to muscle during activation and, typically, while the muscle is forcibly lengthened (Newham et al., 1983; McCully and Faulkner, 1985). Compared with type 1 fibers of the LVP muscle from normal palates, type 2 fibers from nonrepaired cleft palates are more susceptible to lengthening contraction–induced injury (Rader et al., 2006). The magnitude of contraction-induced injury is best assessed by determination of the force deficit, the decrease in maximum isometric force expressed as the percentage of the initial maximum force (McCully and Faulkner, 1985). To determine the susceptibility to injury for LVP muscle fibers of adult goats, a lengthening contraction protocol (LCP) was administered to single permeabilized muscle fibers, fibers with chemically-breached plasma membranes (Rader et al., 2006). The force deficits for fibers from goats with cleft palates were twice as large as the deficits for goats with normal palates (Rader et al., 2006). The conclusion was that,

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at least for adult goats, nonrepaired cleft palates contain a large percentage of type 2 fibers that are more susceptible to lengthening contraction–induced injury than are fibers from LVP muscles of control goats.

Surgical repairs of cleft palates typically involve eliminating the abnormal insertion of the LVP muscle along the posterior edge of the bony palate and realigning the muscle across the posterior soft palate by performing an intravelar veloplasty to reconstitute the normal muscular sling (Randall and LaRossa, 1991). One potential mechanism for achieving velopharyngeal competence after surgical repair is attaining concomitant fiber-type transformation from injury-susceptible type 2 to injury-resistant type 1 fibers. Conversely, persistent velopharyngeal dysfunction after surgical repair may be due, in part, to the failure to realize adequate fiber-type transformation from injury-susceptible type 2 to injury-resistant type 1 fibers. As an initial step to determining whether this theory is valid, we investigated whether a fiber-type conversion and a decrease in the susceptibility to lengthening contraction–induced injury occurs postrepair for most cases. Single permeabilized LVP muscle fibers were obtained from congenitally-clefted palates of goats before and after surgical repair. The testing of permeabilized fibers allowed for the analysis of the behavior of sarcomeres by eliminating the effects associated with the intact plasma membrane (Macpherson et al., 1996). The hypothesis tested was that repair decreases the percentage of type 2 fibers and the susceptibility to injury to levels that are less than those for nonrepaired cleft palates. Evidence supporting the hypothesis would be consistent with the proposal that fiber-type composition and resistance to injury may be important in the development of a functional LVP muscle following surgery.

METHODS

Levator veli palatini muscle fibers were isolated from normal palates of young goats at the time of weaning, 2 months of age. For congenitally-clefted palates, LVP muscle fibers were harvested immediately before surgery (performed at 2 months of age) and 6 months following surgery. The investigators who collected the data, as well as the equipment and the methods used, were the same as described in a previous report regarding LVP muscle fibers from three normal and three nonrepaired cleft palates of 14- to 15-month-old adult goats (Rader et al., 2006). Consequently, these data obtained from adult goats (Rader et al., 2006) were included in the present analyses for comparison purposes.

Congenital Cleft Palate Model

Eleven Spanish goats were used for the study; two young male goats and three adult female goats had normal palates, and three young male goats and three adult female goats had congenital cleft palates. During gestational days 32 to 41 (palatal fusion occurs at gestational day 38), cleft palates were induced by gavaging pregnant goats with an extract of *Nicotiana glauca* plant slurry twice daily (Panter and Keeler, 1992; Weinzweig et al., 1999). The extract causes the neck of the fetal goat to remain hyperflexed, thereby causing the tongue to elevate and obstruct the migration of the palatal shelves to the midline. Thus, palatal fusion is physically inhibited in 97% of cases, with complete clefting of the secondary palate in all cases (Weinzweig et al., 1999).

Cleft Palate Repair and LVP Muscle Harvest

At 2 months of age, cleft palate repair consisted of a modified von Langenbeck technique that included a two-layer repair performed anteriorly along the hard palate and bony cleft using interrupted 3-0 chromic sutures. The soft palate was closed in two or three layers, depending on the consistency of the muscle. Although a formal intravelar veloplasty was not performed, the muscle was repaired by dissecting the LVP muscle from the posterior edge of the maxilla and suturing the muscle to itself across the posterior soft palate. Although no hamulus region

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was observed in the palates of goats during the surgery, the repair occurred in a region analogous to that used in humans (Randall and LaRossa, 1991). General endotracheal anesthesia and intravenous antibiotic prophylaxis (ceftriaxone, 1 gm) were used. Following recovery, all goats were bottle-fed for 5 days prior to returning to nursing, with care taken to avoid injury to the palatal suture lines. Within several weeks, all goats had been weaned from nursing and tolerated a standard pellet diet.

The LVP muscles were harvested from the nonclefted normal palates of the young goats and from the normal and nonrepaired cleft palates of the adult goats in one stage. In contrast, muscles were harvested on two occasions from the three goats that underwent cleft palate repair; tissue was obtained immediately prior to repair at 2 months of age and again 6 months after the repair at 8 months of age. The amount of LVP muscle harvested at the time of cleft palate repair was minimal (20 mg) and consequently did not impact the ability to perform a muscle repair. The procedures were approved by the Harvard Medical Area Standing Committee on Animals and in accordance with the guidelines of the U.S. Public Health Service, NIH Publication No. 85-23.

Muscle Fiber Preparation

Immediately prior to surgery, in the case of the repaired group, or prior to sacrificing, in all other cases, the LVP muscle was harvested. Muscles were transferred into skinning solution composed of 125 mM potassium proprionate, 20 mM imidazole, 5 mM ethyleneglycol-bis (B-aminoethyl ether) tetra-acetic acid (EGTA), 2 mM $MgCl_2$, and 2 mM ATP at 4°C and were separated into bundles. Permeabilization was enhanced by placing the bundles in skinning solution containing Brij 58 (0.5 w/v) at 4°C for 30 minutes. The bundles were transferred into storage solution composed of skinning solution, with glycerol substituted for 50% of the water volume at 4°C for 24 hours. The bundles were then transferred to fresh storage solution and kept at −20°C until the extraction of single fibers was required. To extract single fibers, the bundles were placed into relaxation solution (pCa ~ 9.0) (pH 7.1) composed of 90 mM HEPES, 10.3 mM Mg (total), 1.0 mM Mg^{2+}, 50 mM EGTA, 8.0 mM ATP, 10.0 mM CrP, 1.0 mM NaN_3, 36 mM Na (total), and 125 mM K (total). While in relaxation solution, individual fibers were pulled gently from each bundle using fine forceps.

Morphological Measurements

Each muscle fiber then was placed into a chamber containing relaxation solution at 15°C. The fiber was secured to a force transducer (Model 403A; Aurora Scientific, Inc., Ontario, Canada) and a lever arm of a servomotor (Model 322C; Aurora Scientific, Inc.) using 10-0 nylon suture. Upon setting sarcomere length to 2.5 μm by projecting a laser diffraction pattern produced by the fiber onto a calibrated target screen, the fiber length (L_f) was measured. Two high-magnification digital images were obtained, one image of the top view and another of the side view. For each view, five diameters were measured at 100 μm intervals along the fiber. For each pair of diameters corresponding to an interval, fiber cross-sectional area was calculated assuming an elliptical cross section. The mean cross-sectional area was used to estimate the overall fiber cross-sectional area (CSA). Muscle mass was estimated as the product of L_f, CSA, and the density of 1 mg/mm^3 (Macpherson et al., 1996).

Determination of Resting Force

Each fiber was transferred to a chamber containing a low-[Ca^{2+}] preactivation solution (pH 7.1) composed of 90 mM HEPES, 8.50 mM Mg (total), 1.0 mM Mg^{2+}, 50 mM EGTA, 50 mM Ca^{2+} (total), 8.0 mM ATP, 10.0 mM CrP, 1.0 mM NaN_3, and 36 mM Na (total). The preactivation solution was weakly-buffered for Ca^{2+} so that rapid activation and force development would be possible once submerged in activation solution (Moisescu and Thieleczek, 1978). The fiber was allowed to equilibrate for 3 minutes, at which time the fiber

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was exposed to a transient slack release. The rate of slack release exceeded the maximal velocity of shortening, and consequently, the force during that time period was exclusively a result of passive tension. This force was referred to as the resting force (P_{rest}).

Contractile Properties to Determine Fiber Type and Specific Force

The fiber was activated maximally when moved into a chamber containing activation solution (pCa ~ 4.5) (pH 7.1) composed of 90 mM HEPES, 8.12 mM Mg (total), 1.0 mM Mg^{2+}, 50 mM EGTA, 50 mM Ca^{2+} (total), 8.0 mM ATP, 10.0 mM CrP, 1.0 mM NaN_3, 36 mM Na (total), and 125 mM K (total). Structural stability of the fiber was maintained throughout activation by cycling between an isometric contraction and short periods of isovelocity shortening near the maximal velocity of shortening, followed by a rapid return to initial L_f (Sweeney et al., 1987). To determine fiber type, the fiber was exposed to a slack release followed by a 5-millisecond lengthening step to 105% of L_f and a rapid return to L_f to momentarily unload the fiber. The redevelopment of tension following the unloading was fit using analysis software (Signo; Alameda Applied Sciences, San Leandro, CA) and a least-squares fit to the following equation:

$$P=P_{fmax}[1-\exp(-k_{rf}t)]+P_{smax}[1-\exp(-k_{rs}t)]+P_{res}$$

In this equation, P is tension at time t, P_{fmax} is the maximal tension for the fast component, k_{rf} is the rate constant of the fast component, P_{smax} is the maximal tension for the slow component, k_{rs} is the rate constant of the slow component, and P_{res} represents the residual tension present immediately after the length release and restretch maneuver. The value of k_{rf} or k_{rs} can be used to determine fiber type (Burton et al., 2005; Hanes et al., 2006; Rader et al., 2006). For this study, the value of k_{rf} was used to categorize each fiber with a value equal to or less than 5 s^{-1} indicative of a slow type 1 fiber (Hanes et al., 2006; Rader et al., 2006). The specific force (sP_0; kN/m^2) was determined by normalizing the maximum isometric force (mN) by the CSA (mm^2).

Lengthening Contraction Protocol

While in activation solution, the fiber was lengthened by 40% of L_f at a velocity of 0.5 L_f/s and returned to L_f at 0.5 L_f/s. The mean force during the lengthening contraction and the force when the fiber length was at 40% of L_f were normalized to CSA generating the corresponding values (kN/m^2) of P_{avg} and P_{peak}. The product of P_{avg} and the displacement were used to determine the work done to lengthen the muscle. The work done (J) was normalized by the mass of the fiber (kg). The magnitude of the injury was assessed by the force deficit, the decrease in maximum force following the LCP expressed as a percentage of the maximum isometric force prior to the LCP (McCully and Faulkner, 1985; Rader et al., 2006).

Statistics

All data were expressed as mean ± one standard error of the mean. To account for the clustered structure of the data due to the sampling of multiple fibers from each goat, linear mixed models (LMMs) were fitted to the data (Verbeke and Molenberghs, 2000; West et al., 2007). This type of model allowed for the analysis of clustered data and eliminated the requirement for all of the fiber data to be independent of one another. The LMMs were executed using the PROC MIXED command in SAS (Version 9.1.3; SAS Institute Inc., Carey, NC) and consisted of the fixed effect of the presence of a normal, nonrepaired cleft or a repaired cleft palate and the random effect of the goats. For each muscle fiber, the storage duration and the number of times that the fiber was thawed prior to testing were included in the LMM as covariates when these factors were significant. For the values of CSA of young goats, marginal negative correlations were observed within data of individual goats. Therefore, the LMM was fitted using a

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compound symmetry covariance structure for the random errors in place of random goat effects. For the values of P_{avg}, mass, and P_{peak} of young goats and values of mass for adult goats, the LMM failed to converge. As a consequence, these data were assessed by a general linear model. For the LMMs and general linear models, the estimates for the coefficients of the fixed effect and covariates are displayed in Table 2. A positive coefficient indicates that the factor and outcome measure are correlated positively, whereas a negative coefficient denotes an inverse relationship. When significance was indicated, multiple comparisons were accounted for by Bonferroni adjustment. Under the circumstance that data were not distributed normally, the Kruskal-Wallis test was used. Significance for each statistical test was set *a priori* at $p \leq .05$.

RESULTS

Characterization of Fiber Type

For young goats, the k_{rf} values for fibers from normal palates, $13.3 \pm 1.4\ s^{-1}$, and cleft palates, $15.5 \pm 1.3\ s^{-1}$, were not different (Fig. 1). With the exception of two muscle fibers from normal palates that had k_{rf} values of 3.5 and 5.0 s^{-1}, the values ranged from 7.8 to 33.4 s^{-1} (Fig. 1). This finding indicated that the fibers were predominantly type 2 (Burton et al., 2005; Hanes et al., 2006; Rader et al., 2006). For the adult goats, differences in k_{rf} values were observed between the groups (Fig. 1; $p < .001$). Compared with the values for normal palates, $2.4 \pm 0.1\ s^{-1}$, the values for the nonrepaired cleft palates, $12.2 \pm 0.8\ s^{-1}$, were sixfold greater. With repair, the k_{rf} values, $4.8 \pm 1.4\ s^{-1}$, were less than the values for nonrepaired cleft palates and not different from those for normal palates. Of the 15 muscle fibers from the repaired palates, 3 had k_{rf} values of 8.7 to 20.0 s^{-1}, which are indicative of type 2 fibers. All three of these muscle fibers were from the same goat. In contrast, all 10 of the muscle fibers sampled from the repaired palates of the other two goats had k_{rf} values of 1.4 to 2.9 s^{-1}. The values for these two goats were characteristic of type 1 fibers.

Morphology, P_{rest}, and sP_0

For the muscle fibers from the normal and cleft palates of the young goats, no differences in L_f, mass, and sP_0 were observed (Table 3). Compared with fibers from normal palates of young goats, those from cleft palates were smaller in CSA by 43% ($p = .03$). The values of P_{rest} for the fibers from cleft palates were twofold greater than those for fibers from normal palates ($p = .02$). At adulthood, no differences in morphology, P_{rest}, or sP_0 were observed between cleft palates and normal palates. The values of L_f for repaired palates were marginally (3%) shorter than those for the cleft palate ($p = .02$). Values of sP_0 for the muscle fibers from the repaired palates were 12% less ($p = .0001$) than those for adult normal palates and 11% less ($p = .001$) than fibers for adult cleft palates (Table 3).

Lengthening Contraction Protocol

For the normal palates and cleft palates of young goats, no significant differences in the forces during the LCP or the force deficits following the LCP were observed (Table 3; Fig. 2) For the adult goats, the work required to stretch the fibers from the repaired palates was 10% less ($p = .001$) than that for fibers from normal palates and 18% less ($p = .0004$) than that for fibers from nonrepaired cleft palates (Table 3). The force deficits of the fibers from nonrepaired cleft palates, 44% ± 2%, were twofold greater ($p < .0001$) than those of normal palates, 23% ± 1%, and those of repaired palates, 20% ± 2% (Fig. 2).

DISCUSSION

The purpose of this study was to determine whether cleft palate repair induced fiber-type transformations and decreased the susceptibility to lengthening contraction–induced injury. Muscle fibers were investigated from normal palates and cleft palates of young 2-month-old

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goats and from normal palates, nonrepaired cleft palates, and repaired palates of adult (8 to 15 months old) goats. For normal palates of young goats, 13% (2 of 16) of LVP muscle fibers were type 1 fibers. For cleft palates of young goats, the complete absence of type 1 fibers and the small size, in CSA, indicated that the development of these fibers was altered. This change in development for fibers of cleft palates was consistent with the fiber-type differences that became more pronounced with age. Despite the potential difference in development, the susceptibility of muscle fibers from young goats to contraction-induced injury was independent of whether the palate was normal or clefted. The implication was that the induction of the cleft palate had no substantial effect on the fiber-type composition and susceptibility to injury. The abnormal insertion along the posterior edge of the bony palate (Weinzweig et al., 2002) and consequent atypical loading of the LVP muscle in cleft palates of the young goats also must have had no apparent effects on fiber type and injury susceptibility. The fiber-type data were consistent with results from a previous report regarding the cleft palates of infants (Lindman et al., 2001). For LVP muscles of children born with clefts, 63% of the fibers were type 2 (Lindman et al., 2001). No normal palates of infants were investigated, so whether the majority of the fibers were type 2 due to the developmental stage of the muscle or to the presence of the cleft could not be determined (Lindman et al., 2001). For the young goats in the present study, the type 2 fibers were a result of the goat's developmental stage and were not dependent upon whether its palate was clefted or normal.

Unlike the large population of type 2 fibers and the force deficits of ~40% for normal palates and cleft palates of young goats, all of the fibers from normal palates of adult goats were type 1 and had a twofold greater resistance to lengthening contraction–induced injury. The finding of a predominance of type 1 fibers was in agreement with previous reports for adult goats (Hanes et al., 2006) and humans (Moon et al., 1998; Stal and Lindman, 2000). During maturation, the fiber-type changes likely were due to activation patterns, hormones, and functional demands (Wigmore and Evans, 2002). In contrast to the data for muscle fibers from adult goats with normal palates, fibers from nonrepaired cleft palates in adult goats were all type 2 and incurred twofold greater force deficits. The abnormal loading of the muscle fibers in the nonrepaired cleft palate may have prevented the appropriate maturation of the fibers (Hanes et al., 2006; Rader et al., 2006). When considering the data for young and adult goats together, regardless of age, the fibers from cleft palates were predominantly type 2 with a twofold greater susceptibility to damage relative to fibers from normal palates of adult goats.

For the muscle fibers from nonrepaired cleft palates of adult and young goats, the maintenance of sP_0 indicated that the fibers were not damaged prior to the LCP (Macpherson et al., 1996). Because no evidence of prior injury or myopathy was evident, the differences in the susceptibility to injury were due most likely to fiber-type differences. For limb skeletal muscles of rabbits (Lieber et al., 1991), rats (Macpherson et al., 1996), and humans (Friden et al., 1983, 1988), type 2 fibers are more susceptible to lengthening contraction–induced injury than type 1 fibers are. The differences in the susceptibility to injury may be a result of shorter, stiffer ultrastructural proteins for type 2 fibers compared with those of type 1 fibers (Agarkova et al., 2004; Prado et al., 2005). Therefore, the high susceptibility to LCP-induced damage at the time of repair for young goats and for the muscle fibers from nonrepaired cleft palates of adult goats was most likely a result of the high percentage of type 2 fibers.

Surgical repairs of cleft palates are designed to be beneficial but of necessity are invasive. As a consequence, they have the potential for both advantageous and disadvantageous effects on palatal muscle (Randall and LaRossa, 1991). The values of sP_0 for the muscle fibers from repaired adult palates were ~10% less than those from age-matched normal palates and cleft palates. The decrease in sP_0 was a result of malfunction either in the form of a reduction in the number of cross-bridges per unit area, fewer cross-bridges in the driving stroke at any particular time, or a decrease in the force production per cross-bridge (Brooks and Faulkner, 1988). The

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implication was that during or following surgical cleft palate repair, LVP muscle incurred some long-term damage. Despite the ~10% decrease in sP_0 with repair, the fibers were predominantly type 1 and resistant to lengthening contraction–induced injury. Postrepair, muscle fibers incurred LCP-induced force deficits that were half of those for age-matched nonrepaired cleft palates. This decrease in injury with repair was accompanied by a decrease in values of work during the LCP. The magnitude of lengthening contraction–induced injury is a function of the work required during the stretching (Brooks and Faulkner, 1996). For muscle fibers of mice, the force deficit increased by 10% with every 20% increase in work (Brooks and Faulkner, 1996). In the present study, the fibers from repaired palates had work values that were only 10% less than those for fibers from nonrepaired cleft palates. Therefore, the difference in work values was unlikely to be a major factor responsible for the force deficit differences. Rather, the fiber-type changes that accompanied repair potentially account for the dramatic decrease in force deficit (Macpherson et al., 1996). When considering this finding along with the sP_0 data, the implication is that the fibers were sufficiently adaptable to recover ~90% of their force-generating capabilities postrepair and to convert to type 1 fibers that are resistant to LCP-induced injury. The alterations in the orientation of the muscle fibers upon repair resulted in a different loading pattern and likely provided the environmental cues necessary for fiber-type conversion (Wigmore and Evans, 2002).

Variability in the responsiveness to repair was observed among the goats. All of the fibers for two of the goats were type 1. For the third goat following repair, three of the five fibers tested were type 2. These type 2 fibers incurred high force deficits of ~30% following the LCP. The finding that values of CSA for these type 2 fibers were half of those for type 2 fibers from nonrepaired cleft palates implies that the fibers may have been rarely activated and loaded, factors important for the determination of fiber size (Timson, 1990; McKoy et al., 1999; Hornberger et al., 2005). The observation of type 2 fibers in only one of the goats may have been a result of the limited number of goats available for study. The cost and time required for generating each goat model meant that testing fibers from only a small number of goats per group was feasible. Another explanation for the outcome of type 2 fibers in only one of the goats may be that a minority of LVP muscles do not respond as well to cleft palate repair. Therefore, this finding may have implications for why 15% of patients are unable to achieve velopharyngeal closure following repair (Marrinan et al., 1998).

The LCP was designed so that the differences in the susceptibility to injury between the groups could be quantified. For comparison purposes, even the most injury-resistant type 1 muscle fibers had to be injured to some extent. The type 1 muscle fibers from the hind limbs of rats required a lengthening contraction of 40% strain for these fibers to sustain a 10% force deficit (Macpherson et al., 1996). As a consequence, the same strain was chosen for the present study. The protocol was not selected to simulate the typical contractions of the LVP muscle. During talking, the LVP muscle usually is exposed to shortening contractions rather than lengthening contractions (Ettema et al., 2002). The extent to which the LVP muscle is exposed to injurious events during or following surgery and whether these injuries have similar features to lengthening contraction–induced injury remains to be investigated. The results of the present study demonstrated that, at least following the LCP tested, muscle fibers from cleft palates of goats are predominantly type 2 and are highly susceptible to injury, even at a young age. In addition, surgery at a young age was effective at decreasing the percentage of type 2 fibers and the susceptibility to injury. Because fiber-type transformations appear to be necessary for changes in injury susceptibility, perhaps the success of repair is dependent on age. The implication then would be to repair palates earlier to promote fiber-type conversions to type 1 fibers. Further investigation of the factors that predispose muscle fibers to convert to type 1 fibers following surgery may help to decrease the percentage of type 2 fibers that remain and improve the incidence of functional palatal movement.

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Acknowledgments

This project was supported by a 2005 American Society of Maxillofacial Surgeons / Maxillofacial Surgeons Foundation Synthes CMF Research Grant Award. The single muscle fiber preparation and testing was funded by the Nathan Shock Center of Excellence in Basic Biology of Aging Grant P30 AG 13283. E. Rader was supported by a fellowship, the National Institutes of Health (NIH) Regenerative Sciences Training Grant T90 DK-070071. D. Yu had financial assistance from the NIH Training Grant in Trauma, Burn, and Wound Healing Research Grant T32 GM008616-06A1. P. Cederna was an Academic Scholar of the American Association of Plastic Surgeons.

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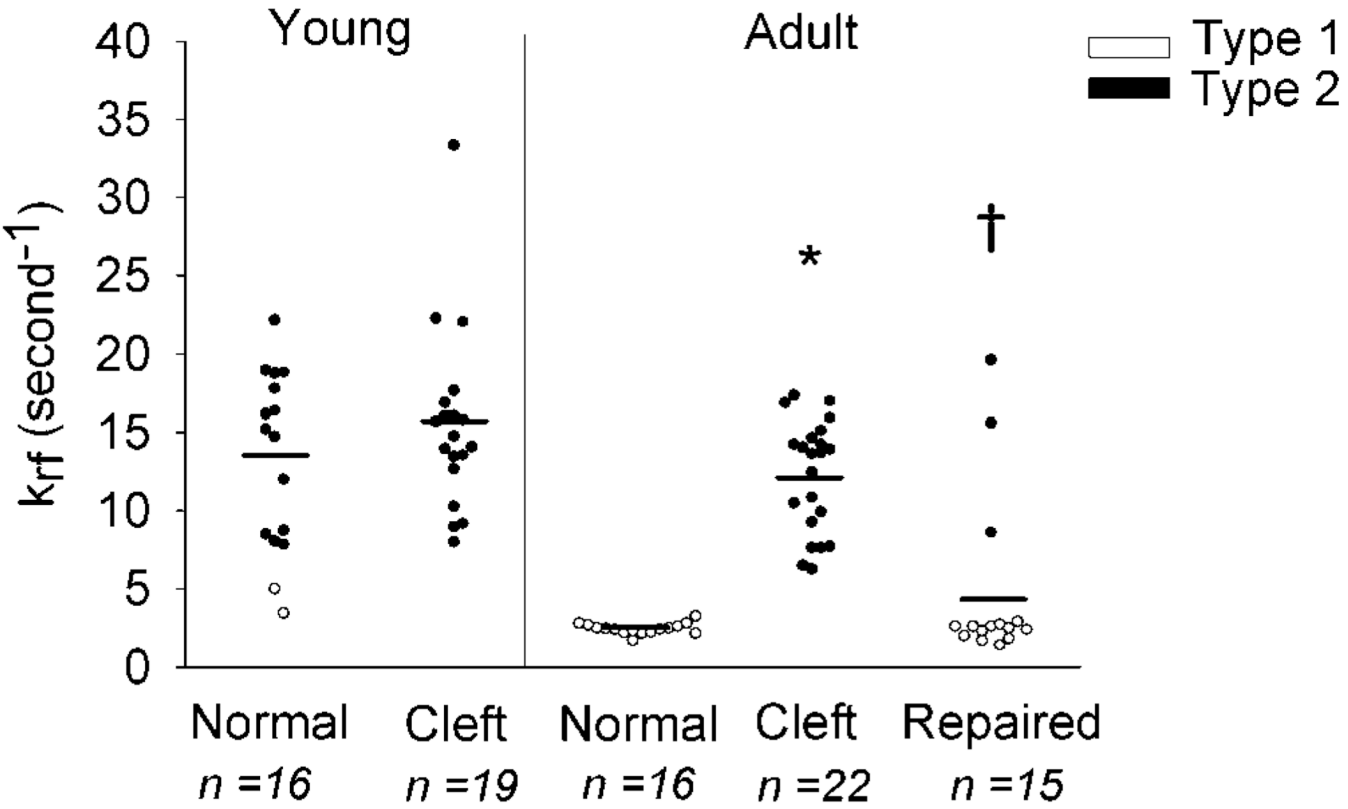

FIGURE 1.
Values of k_{rf} for muscle fibers from normal, nonrepaired cleft and repaired cleft palates of goats following the LCP. Each circle represents one fiber and the bars denote mean values. * Different ($p \leq .05$) from value for age-matched normal palates. † Different ($p \leq .05$) from value for age-matched cleft palates.

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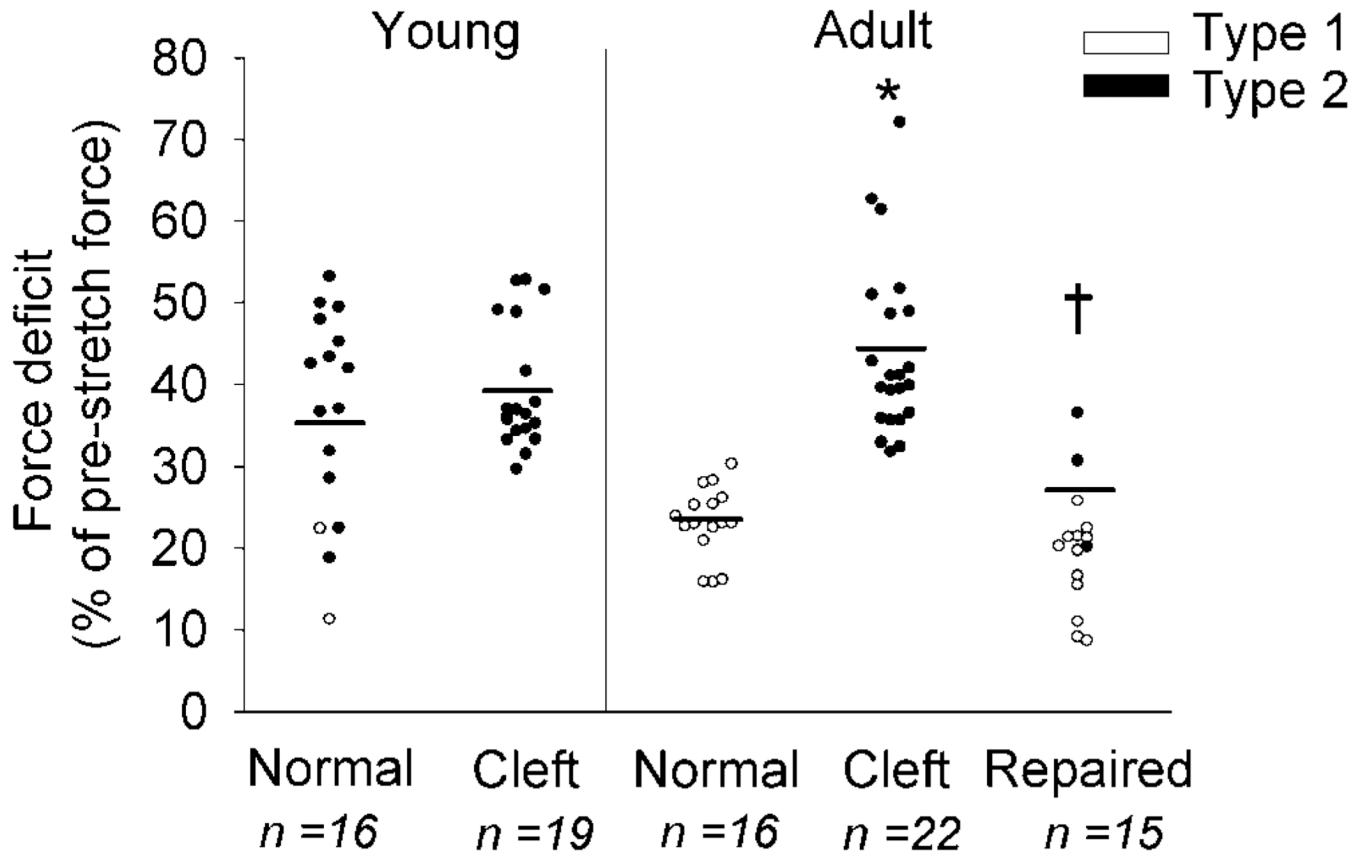

FIGURE 2.

Force deficits for muscle fibers from normal, nonrepaired cleft and cleft repaired palates of goats following the LCP. Each circle represents one fiber and the bars denote the mean values. * Different ($p \leq 0.05$) from value for age-matched normal palates. † Different ($p \leq .05$) from value for age-matched cleft palates.

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TABLE 1

Abbreviations

LVP	Levator veli palatini
VPI	Velopharyngeal incompetence
MHC	Myosin heavy chain
LCP	Lengthening contraction protocol
L_f	Resting fiber length
CSA	Cross-sectional area
P_{rest}	Resting force
k_{rf}	Rate constant of fast component for force redevelopment
sP_o	Specific force
P_{avg}	Mean force during the lengthening
P_{peak}	Force when fiber was maximally lengthened
LMM	Linear mixed model
P_{post}	Force following lengthening

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TABLE 2

Coefficients for the Fixed Factors of the Statistical Model for Each Experimental Measure* (see Table 1 for explanation of column headings)

	L_f (mm)	CSA (μm^2)	Mass (μg)	P_{rest} (kN/m^2)	P_{rest} (kN/m^2)	P_{avg} (kN/m^2)	P_{peak} (kN/m^2)	Work (J/kg)	P_{post} (kN/m^2)	Force Deficit (%)	k_{rf} (s^{-1})
Young											
Cleft palate	0.167 ± 0.158	−433.32 ± 116.15	−0.50 ± 0.23	6.65 ± 2.62	−15.22 ± 12.73	−18.33 ± 14.54	−5.36 ± 14.60	−5.36 ± 3.39	−13.35 ± 7.83	NA	1.62 ± 2.94
Number of prior thaws	—	—	—	1.84 ± 0.76	—	—	—	—	−10.12 ± 4.04	NA	—
Storage duration in days	—	—	—	—	−0.18 ± 0.04	−0.15 ± 0.04	−0.13 ± 0.04	−0.06 ± 0.01	−0.10 ± 0.02	NA	—
Intercept	1.598 ± 0.116	1326.56 ± 96.38	2.12 ± 0.20	4.57 ± 2.11	178.90 ± 13.74	271.68 ± 15.63	284.62 ± 15.70	108.75 ± 3.61	116.50 ± 8.35	NA	13.27 ± 2.23
Adult											
Cleft palate	0.026 ± 0.095	204.51 ± 297.37	0.23 ± 0.55	0.62 ± 1.95	13.07 ± 8.45	23.70 ± 12.23	30.33 ± 17.07	10.07 ± 4.12	NA	12.96 ± 3.57	NA
Repaired cleft palate	−0.229 ± 0.095	−158.27 ± 296.69	−0.64 ± 0.48	1.50 ± 1.91	−22.77 ± 3.68	−20.35 ± 8.69	−10.50 ± 17.92	−7.29 ± 1.89	NA	2.11 ± 3.51	NA
Number of prior thaws	0.062 ± 0.018	—	—	2.47 ± 0.41	−7.07 ± 1.60	—	—	—	NA	3.49 ± 0.75	NA
Storage duration in days	0.002 ± 0.001	—	—	0.03 ± 0.01	—	—	—	—	NA	0.04 ± 0.02	NA
Intercept	1.598 ± 0.130	2160.27 ± 175.73	4.09 ± 0.33	−0.75 ± 2.51	116.43 ± 3.83	207.08 ± 6.24	±12.55	82.50 ± 1.30	NA	8.51 ± 4.60	NA

*Values are estimates of the coefficients ±1 standard error; em dashes indicate that the effect of prior thaws or storage duration did not reach a level of significance (.05) necessary for inclusion in the statistical model; NA = not applicable because the data were not distributed normally and, consequently, were analyzed by a nonparametric test that did not generate estimates of coefficients.

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TABLE 3

Data Before and During Lengthening Contraction Protocol to Single Permeabilized Fibers From LVP Muscles of Normal, Cleft, and Repaired Palates of Goats[‡] (see Table 1 for explanation of column headings)

	n	L_f (mm)	CSA (μm^2)	Mass (μg)	P_{rest} (kN/m^2)	sP_o (kN/m^2)	P_{avg} (kN/m^2)	P_{peak} (kN/m^2)	Work, J/kg	P_{post} (kN/m^2)
Young										
Normal	16	1.59 ± 0.1	1321 ± 117	2.1 ± 0.2	6 ± 1	131 ± 12	230 ± 13	248 ± 11	92 ± 5	81 ± 7
Cleft	19	1.78 ± 0.1	881 ± 50[*]	1.6 ± 0.1	13 ± 1[*]	92 ± 7	192 ± 9	225 ± 10	77 ±4	56 ± 4
Adult										
Normal	16	1.88 ± 0.1	2152 ± 133	4.1 ± 0.3	9 ± 1	105 ± 5	207 ± 7	225 ±8	83 ±3	81 ± 4
Cleft	22	1.78 ± 0.1	2363 ± 205	4.3 ± 0.4	16 ± 2	103 ± 9	231 ± 11	257 ± 11	92 ±4	60 ± 6
Repaired	15	1.72 ± 0.1[†]	2002 ± 213	3.4 ± 0.4	7 ±1	92 ± 3[*†]	187 ± 6[†]	215 ±8	75 ± 2[*†]	74 ± 3

[*] Different ($p \leq .05$) from value for age-matched normal palates.

[†] Different ($p \leq .05$) from value for age-matched cleft palates.

[‡] Values are means ± 1 standard error of the mean; n = number of fibers.