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Response of hypotrophic muscles to altered skeletal morphology and functional rehabilitation

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RESPONSE OF HYPOTROPHIC MUSCLES TO ALTERED SKELETAL MORPHOLOGY AND FUNCTIONAL REHABILITATION

by

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THESIS

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I would like to dedicate this thesis to my wife, Linda. Only I will ever know the many sacrifices she made in supporting me during the creation of this manuscript. Her love and her caring helped keep me going when the going got rough. I want her to know how truly thankful I am for her support.

The Author

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INTRODUCTION

A normal neuromuscular system has a large capacity to adapt to functional changes. This research will characterize the ability for congenitally hypotrophic muscles to develop in response to altered skeletal morphology and functional rehabilitation. Do such muscles have the ability, with skeletal reconstruction and functional rehabilitation, to increase their size and density in excess of their congenital development? A system in which this can be studied is provided by hemifacial microsomia patients. Surgical correction of their congenital hard tissue deficiences presents an excellent opportunity to study muscle development in response to altered skeletal morphology and function.

In order to test our hypotheses the relatively new application of computed tomography (CT) analyses to measure soft tissue changes needed to be developed and standardized. This is a relatively new area. To date, a standardized method of measuring soft tissue changes over time using CT does not exist.

A. Review of the Literature

1. Skeletal Muscle Physiology

Many aspects of skeletal muscle's adaptive capabilities have been studied in the past and merit review. A case in point is the ability and conditions under which a free muscle end can attach to bone, a major first step in muscle development. A second example is the possibility of muscle attachment to an actively remodeling bony surface, particularly important considering that this study makes use of bone grafts in the correction of the hard tissue deficiencies of hemifacial microsomia patients. Lastly, since this paper is exploring skeletal muscle growth and development in general, it is appropriate to review background material in this area as well.

In order for a muscle to function and develop properly, it must first have an origin and insertion. Muscles with detached free ends have been shown to reattach to bony surfaces. In a histological study, Choulkas and others (1968) reported that masseter muscles which had been surgically detached from rat mandibles showed evidence of reattachment to bone in as little as 48 hours. In their experiment, the lower two-thirds of the muscle was first reflected superiorly from its bony attachment. The reflected muscle was then loosely replaced on the bony surface in its normal position and only the skin was sutured in place. After two days, histological sections revealed that the masseter was still separated from the mandibular ramus by a blood clot in all regions with the exception of at the lower border. Here, tendinous slips could be seen penetrating the periosteum and were embedded in the cortical plate. Muscle fibers on the lateral surface of the mandible were seen reattached to the periosteum but they did not penetrate the bone. Four days post-surgery proliferation of the periosteum and bone apposition was evident on the mandibular inferior border. At one week the new bone at the lower

border of the ramus consisted of projecting spicules enclosing loose connective tissue. In addition, multinucleated cells could now be found aggregated upon the surface and between the muscle bundles. These cells formed myotubes as the result of the fusion of myoblasts. This is the initial step in new muscle fiber formation. When healing was finally complete, the muscle fibers were embedded in the bony surface at the inferior border by way of tendons. Upon the ramus surface, the muscle fibers terminated by fibrous connective tissue at the periosteum. Furthermore, striated muscle tissue was noted in the nascent myotubes three weeks post-surgically.

Chierici and Miller (1984) performed a similar study in rhesus monkeys in which the temporalis muscle was detached at its origin. This study helped elucidate some of the conditions under which muscle reattachment takes place. Two weeks post-operatively they observed new bone spicules developing from the surface of the bone that was oriented in the direction of the detached muscle fibers. This new bone formed before attachment was reestablished. Furthermore, the bony spicules only formed at the future attachment site, a short distance from the free muscle ends, rather than beneath the fiber bundles that course over the bony surface. Their study concluded that a detached muscle which remains in close proximity to a bony surface will become reattached by new bone formation which encloses the ends of muscle fibers and tendon. They did not establish, however, how far apart bone and muscle endings could be and still achieve reattachment. They did show that the processs can occur when a distance of two to three millimeters between the bony surface and detached muscle endings exists.

To date, there is no histological study of the new attachment of vestigial muscles to bone or a bone graft. This may or may not be qualitatively

different from a situation in which a normal muscle reattaches to a bony surface that was previously an attachment site.

As mentioned above, bone grafts play an important role in this study. One important characteristic pertaining to bone grafts is that the graft is actively resorbed and remodeled soon after its placement. An experimental study by Hoyte and Enlow (1966) has shown that this should not, however, prevent muscle attachment from occurring. They demonstrated that muscle attachment can occur even in areas of bony surface resorption during hard tissue remodeling.

The growth and development of skeletal muscle is multifaceted. In adults, this response is primarily due to muscular hypertrophy (Goldberg et al., 1975) since the adult number of multinucleated skeletal muscle cells in humans is attained before birth (Goldspink, 1974). Postnatally, increase in muscle size can occur in one of three ways. Two of these mechanisms fall into the category of cell enlargment. First, muscles can increase in length. This is as a result of recruitment and fusion of myoblasts at muscle fiber ends (Goldspink, 1974) or from addition of sarcomeres to the ends of myofibrils (Williams and Goldspink, 1971). Second, muscle hypertrophy can occur by an increase in its girth, i.e. its cross-sectional dimension. This is actually an increase in the size and number of contractile myofibrils within each muscle cell, commonly seen in weightlifters (Goldspink, 1974). Third, some muscle hyperplasia is possible. Myoblasts known as satellite cells which are present on the surface of muscle fibers but within the muscle's basal lamina can proliferate and fuse to form new fibers. Fibroblast growth factor has been shown in culture to be a key factor in helping to activate these myoblasts (Bischoff, 1986).

2. Hemifacial Microsomia (HFM)

Patients with HFM can present with a nearly unilateral deformity involving malformation of the mandible and underdevelopment or even absence of one or more of the four muscles of mastication on the affected side. HFM is a variable, congenital craniofacial deformity involving structures of the first and second branchial arches. It most commonly presents as a unilateral underdevelopment of the external ear, the middle ear ossicles, the condyle and ramus of the mandible, the zygomatic arch, the malar bone, and the squamous portion of the temporal bone. Bilateral cases are seen, but there is usually a considerable difference in the expression of the syndrome between the two sides. The muscles of mastication and facial expression are affected to a variable extent. Macrostomia and seventh cranial nerve paresis may also be found.

Although the mechanism in humans is unknown, Poswillo (1973) developed animal models with defects similar to HFM by administering thalidomide to *Macaca irus* monkeys and triazene to CS1 mice. These defects were most likely induced by an embryonic hemorrhage arising from a leak in the developing anastomosis of those vessels that proceed to form the stapedial arterial stem. These vessels are the ventral ascending pharyngeal and hyoid arteries, which anastomose over the second branchial groove. The hemorrhage produced a focal hematoma between the first and second branchial arches of the mice during a critical period in morphogenesis, resulting in a sequential process of destruction, repair and redifferentiation. The varying extent of the hematoma could explain the wide spectrum of variation seen in HFM.

Teratogens are believed to be one cause of embryonic hemorrhages. The incidence of spontaneous defects of the first and second branchial arches

in humans is about 1 per 4,000 live births. In Germany, following administration of thalidomide to women in the first six weeks of pregnancy from 1959 to 1962, at least 3,000 cases of defects of the first and second branchial arches were observed (Poswillo, 1973).

HFM patients are classified by the amount of mandibular deformity present. However, this type of classification has not been standardized and different investigators have used different schemes (Harvold, 1975, Kaban et al., 1981, Munro, 1987). The Center for Craniofacial Anomalies at the University of California at San Francisco classifies the various mandibular deformities into five different types (see Table 1), (Chierici, 1983). In an effort to demonstrate the most dramatic changes and have a sample of patients who receive a similar surgical procedure, this study focuses on HFM patients with the most severe types of deformity. HFM patients with type IV mandibles (Figure 1a) have only a coronoid process of varying size present on the affected side; there is no condylar process or glenoid fossa and, consequently no temporomandibular joint structures. There may or may not be an articulating surface. Rotational movement allows jaw opening, but there is no forward translation of the affected side. HFM patients with type V mandibles (Figure 1b) are missing the entire ramus, including the coronoid and condylar processes on the affected side. The body of the mandible ends with the enclosure of the last tooth bud. There is a wide separation between the end of the mandible and the temporal bone. The four muscles of mastication (temporalis, masseter, medial and lateral pterygoids) in both these types of patients are underdeveloped or even missing on the affected side. If present, the muscles may be fragmented and interspersed with adipose and connective tissue. Some type V patients have been described as missing three of the four

Table 1: HFM classification based on the type of mandibular deformity, the University of California at San Franscisco Center for Craniofacial Anomalies System (Chierici, 1983).

HFM Type	Description
I	Absence of condylar cartilage and disc
II	No condylar head or neck on condylar process
III	Ankylosis or syndesmosis of temporomandibular joint
IV	Coronoid process present, condylar process missing
V	Both coronoid and condylar processes missing

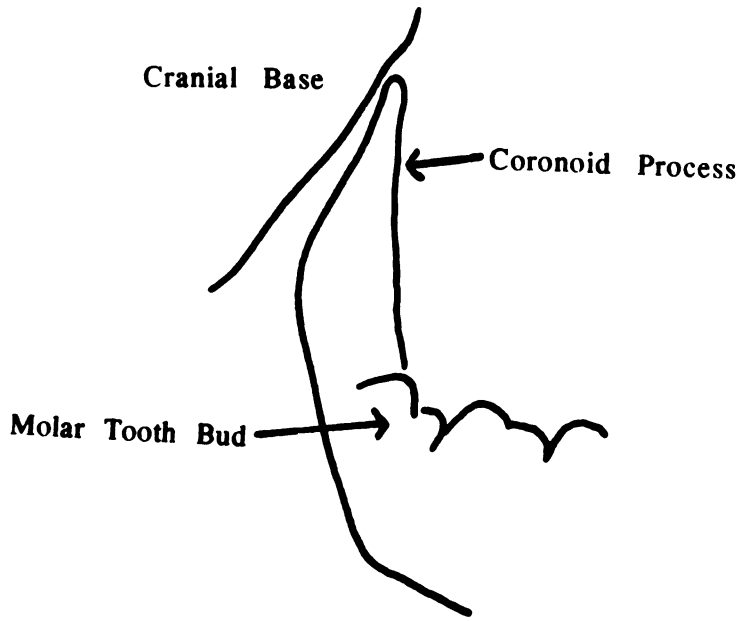


Figure 1a: Type IV HFM mandibular deformity. Note the absence of a condylar process.

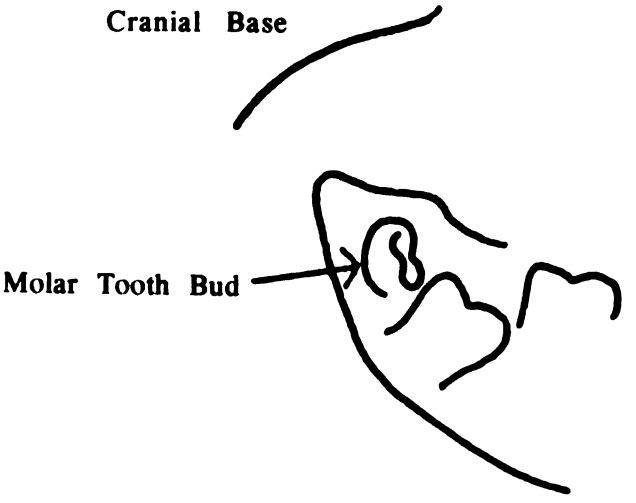


Figure 1b: Type V HFM mandibular deformity.
The ramus is missing and the mandible ends behind the last developing molar.

masticatory muscles on the affected side with only the temporalis muscle being detected by EMG recordings (Vargervik and Miller, 1983).

There are a number of different treatment approaches regarding timing, management philosophy, and surgical procedures utilized in HFM (Obwegeser, 1974, Poswillo, 1974, Edgerton and Marsh, 1977, Harvold, 1975 and 1983, Mulliken and Kaban, 1987, Munro, 1987, Kaban, 1990). Most of those cited agree that early treatment including early surgical intervention should be performed in severe cases of HFM.

Treatment of type IV and V HFM patients at the UCSF Center for Craniofacial Anomalies includes several phases. First, a functional appliance is used to improve lower jaw position and free the maxilla, thus, decreasing the mandible's detrimental effects on the maxilla such as canting of the maxillary occlusal plane (Vargervik, 1983b). According to Harvold, the orthopedic appliance can also serve as a substitute for the missing joint-muscle mechanism in HFM. "This repositioning carries the abnormal ramus forward, resulting in remodeling and increase in size on both the abnormal condylar and coronoid processes," (Harvold, 1983). Timing of the surgical reconstruction of the deformed mandible is usually based on skeletal findings, dental development, response to treatment, and psychological factors (Vargervik, 1983a). The surgical procedure consists of ramus construction utilizing a costochondral or iliac crest bone graft (Figure 2). A soft tissue space is created for the bone graft from the end of the mandible to the floor of the skull in the area of the absent glenoid fossa. The mandible is freed from all muscle and ligament attachments. Next, the rectangular graft is wired to the mandible and placed against the cranial base. Construction of a temporomandibular joint may or may not be attempted (Ousterhout and Owsley, 1983).

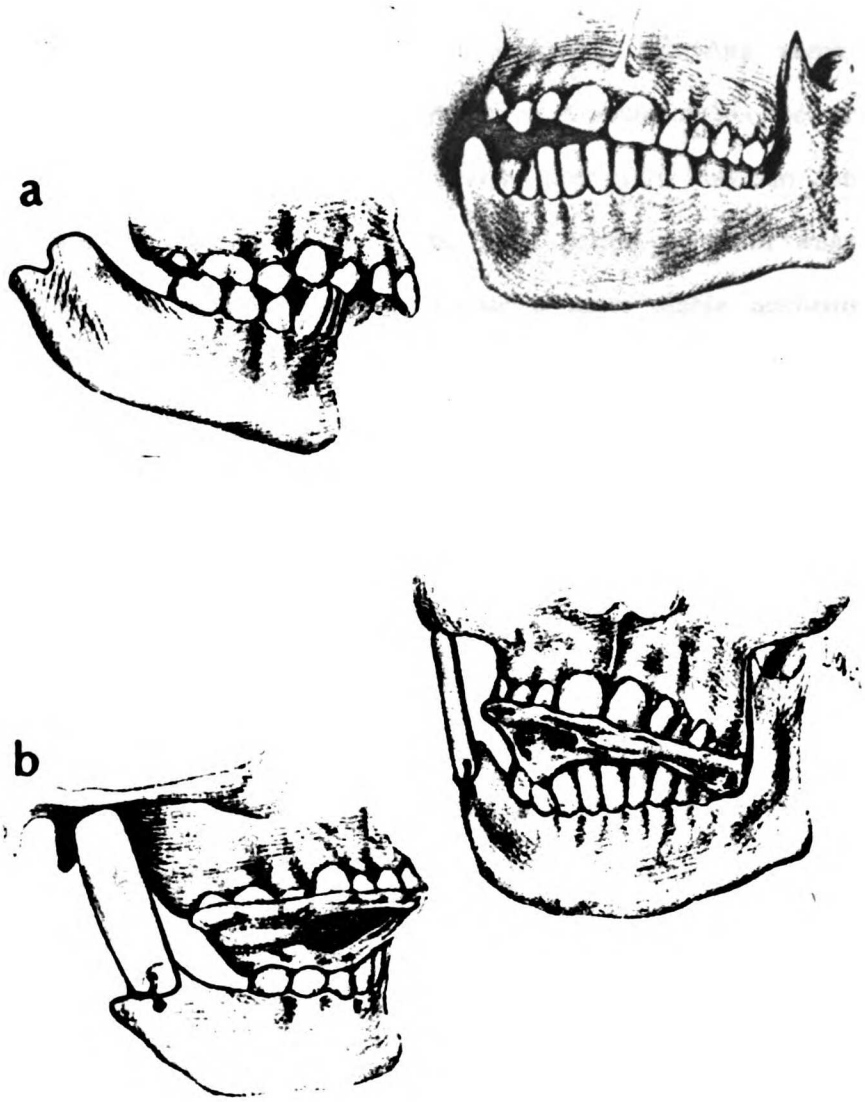


Figure 2: (A) Type V HFM prior to surgery. (B) Surgical reconstruction of the mandibular ramus using a bone graft.

Following the operation, the patient is placed in intermaxillary fixation (IMF) for 8 weeks with an occlusal splint which adds stability and maintains the surgically created posterior open bite on the affected side. After IMF, the splint is just attached to the maxillary arch with rubber bands and the lower jaw is allowed to function against the splint. Light rubber bands are placed between the maxillary and mandibular archwires allowing some jaw movement and protecting the graft matrix. Concurrently, the graft is being resorbed and remodeled. Radiographically, the graft may no longer be distinguishable in as little as 5 months. The period of splint wear lasts about 6 to 9 months, until orthodontics can create a more stable occlusion by extruding the teeth on the affected side to contact with the mandibular teeth. This rehabilitational phase is extremely important in determining long term bone morphology (Vargervik, 1985, Vargervik et al., 1986).

As mentioned previously, a detached muscle which remains in close proximity to a bony surface can become reattached by new bone formation which encloses the ends of muscle fibers and tendon (Chierici, 1984). This process can occur even if the end of the detached muscle remains a short distance from the bone surface. As neuromuscular functions are restored, areas of both the graft and new bone will resorb until the surface conforms to the perimeter of the tension zone created by the soft tissue environment. It is hypothesized that changes are occuring concomitantly in the newly attached muscles. Thus, in the treatment of HFM patients we have a model in which to study the adaptive response of muscles to structural and functional changes.

3. Computed Tomography

With the aid of computed tomography (CT) scans, it is possible to measure certain parameters of soft tissues changes from one time point to the

next (Cann, 1983). CT allows display of cross-sectional slices of anatomy, isolating regions from surrounding tissues. The information that is actually recorded by CT detectors is differences in x-ray attenuation. Thus, a CT image is a pictorial map of x-ray attenuation values. The map is stored in digital form in a computer. Images are displayed using a sixteen level gray scale, each attenuation value or range of attentuation values being assigned to one of the levels. With the help of a region-of-interest software program, one can outline areas on an image and measure their size. The attenuation values are a function of atomic number and tissue density; thus, the same region-of-interest software program can calculate the mean density of a particular region. Using known density values of homogeneous standards, one can calculate the actual tissue composition of a specified region from its measured density. This technology is known as quantitative computed tomography (QCT) (Cann, 1983).

QCT has been used to analyze a number of tissue types. Quantitative analyses of bone mineral content and tissue calcification in hard tissue have been performed (Genant and Boyd, 1977, Cann et al., 1982). Other investigators have employed QCT to examine the anatomy and composition of soft tissue. Fike and colleagues (1982a) demonstrated that QCT densitometry studies of the canine brain provided information about normal morphology and physiology, and could be used to evaluate changes in blood flow and blood volume in the brain following therapy.

Densitometry has also been shown to provide quantitative information about fatty infiltration of the liver (Goldberg, 1979). This study showed that a rise in hepatic triglyceride content caused a proportional decrease in CT number values. Based on this relationship and the ability to estimate the normal CT value of liver (by measuring the attenuation value of the spleen) it

was possible to quantify the fat content of the liver. For reference, adipose tissue has a CT value of -100 Hounsfield Units (HU) which is equivalent to a density of 0.9 g/cm^3. Normal muscle ranges in CT value from about 50 to 70 HU, the equivalent densities being 1.05 to 1.07 g/cm^3 (Cann, personal communication). The literature reveals that CT has also been applied to measuring the size of human jaw muscles, however, the methodology is not yet standardized (Gionhaku and Lowe, 1989).

An important concern that arises with the use of CT as a measurement tool is the variability that is present between and within observers as quantitative measurements are made. Friedman et al. (1983) found the reproducibility of two observers' repeated volume determinations of 17 different tumor scans to have a mean difference of 3%, with a range of 0.1 to 17.0% of total volume. Intra-observer variability measurements on canine brain ventricular volumes have been shown to have a coefficient of variation (standard deviation divided by the mean) of 3.03% (Fike et al., 1982b). Previous pilot work done by Miller and Cann (unpublished) in monkeys has demonstrated that the reproducibility of CT measurement for most muscles of the face is about three per cent and that one can quantify changes in volume in response to neuromuscular stimulation in the range of six to seven per cent. These variations can be explained in part by technical problems (see below) as well as observer biases, for example, a tendency to outline structures either on or just inside their borders.

As with any technology, there are some technical problems to be overcome with the use of CT. Since the field is relatively new, there is very little standardization. Thus, the parameters which control how a CT image is viewed need to be considered (Koehler et al., 1979). Specifically, one can alter how the CT images are displayed by changing the CT window and level settings.

This, in turn, can affect the size and shape of these structures as they are examined by an observer. While this has no effect on the data stored in the computer, these parameters should therefore be standardized throughout a study using CT. Another factor to take into account in a long term research QCT study is that a CT scanner undergoes very slight changes over time in what might be termed machine-related instabilities. These changes can be primarily attributed to software updates and variations in the clinical environment. Thus, to obtain high accuracy and reproducibility in a long term research QCT study, one needs to examine QCT data of calibration standards scanned periodically over time and adjust one's own data if need be accordingly (Cann et al., 1985). Another problem related to the use of CT which should be mentioned is radiation exposure. Although the level is quite low, safety dictates that scans should not be performed on the same patient too frequently. This, in turn, restricts the amount of data that can be collected during a long term study using this method. One other technical problem experienced was the difficulty in identifying abnormal muscles on CT scans. While skeletal muscles are easily identified in normal patients, the affected muscles of HFM patients are more challenging to locate due to the fact that they are fragmented and interspersed with adipose tissue. This necessitates that examiners be well-trained in human head and neck anatomy. Furthermore, a long (approximately six months) learning curve of understanding normal and abnomal CT images had to be overcome.

B. Purpose

In this thesis, we hypothesized that the congenitally malformed muscles of HFM patients will/will not increase in size and density in response to therapy. To address this question, quantitative computed tomography is used for the first time. To resolve the thesis question and to calibrate QCT to address the question, the objectives were as follows:

1. Since this kind of research is relatively new, it was important to first develop and standardize a methodology which could be used to gather information on muscle development and test our hypotheses. Thus, the methodology itself needed to be perfected and tested with respect to its ability to provide reliable measurements.

2. To date, the soft tissue asymmetry seen in HFM has only been described qualitatively. A goal of this research was to quantify the side to side variation that exists in HFM, hence, providing hard data that HFM has resulted in asymmetric growth.

3. A third goal of this study was to assess if the treatment of Type IV and V HFM, specifically surgical reconstruction of the mandible and approximately 12 to 18 months of functional rehabilitation, significantly increased the size and the density of the four muscles of mastication on the affected side as compared to the contralateral side. The specific changes that were measured fell into four categories: muscle volume, cross-sectional area, length and density. The parameters of muscle volume, cross-sectional area, and length allow one to measure changes in the overall size of a muscle. The recorded density of each muscle by QCT, on the other hand, is a measure of the tissue composition of the muscle. For example, an increase in density would indicate an increase in the ratio of muscle fibers to adipose tissue.

4. Another goal of this thesis was to assess the variation in side to side masticatory muscle size and density in a control population and to compare this variation to that observed in HFM patients prior to treatment. Furthermore, examining the effects of skeletal reconstruction and rehabilitation toward normalization of the hypotrophic muscles of the HFM affected side was an additional objective. This post-treatment normalization was defined as change in size and density of the affected muscles to values which were closer to those of the control population.

5. Lastly, since this study is the first of its kind to examine HFM patients using CT, a final goal was to describe interesting qualitative findings found among these patients.

C. Hypotheses

This thesis has four major components which are referred to throughout the sections. Part I is concerned with standardization of the methods. Parts II and III deal with the control material and the HFM material, respectively. Part IV examines how the HFM subjects compare with the control subjects. The specific hypotheses that were tested in each part are as follows:

Part I: Standardization of Methods

1. Inter- and intra-observer QCT measurement variability can be decreased through standardization of QCT technology.

Part II: Control Population

1. Side to side variation in size and density of the muscles of mastication in a control population is zero.

Part III: HFM Population

1. The pre-treatment volumes of each of the muscles of mastication on the affected side in HFM are less than those of the contralateral side.

2. Surgical reconstruction of the mandible and rehabilitation will increase the size and density of affected side muscles as compared to muscles of the contralateral side.

Part IV: HFM Subjects vs. Control Subjects

1. Prior to HFM therapy, the size and density of the muscles of mastication on the contralateral side in HFM are similar to the muscles of mastication of a control population.

2. The side to side variation in size and density of the muscles of mastication of pre-treatment HFM subjects is greater than that of control subjects.

3. The HFM muscles' side to side variation in size and density will decrease as a result of treatment, approaching the control population values.

4. HFM therapy will increase the size and density of the affected, hypotrophic muscles of HFM, such that these values will become closer to those values seen in controls.

METHODS AND MATERIALS

A. Part I: Methodology Development

1. CT Scan Protocol and Measurement Parameters

The protocol for the HFM subjects consisted of obtaining CT scans using a GE CT 9800 body scanner at the UCSF Research CT Scanner Facility just prior to their reconstructive mandibular surgery (pre-treatment) and approximately twelve to eighteen months after mandibular repositioning (post-treatment). This time interval allowed for healing and subsequent function.

The CT scans were done using a rapid sequence scanning protocol. The protocol has undergone some modifications over the years. Initially, the protocol consisted of 3.0 mm contiguous transverse slices superiorly and inferiorly and 1.5 mm slices in between, covering 90-120 mm in a total scanning time of about 4.5 minutes to minimize patient motion errors and radiation dose. However, it was difficult to determine just where to stop one slice thickness size and start another, so ultimately a protocol using only 1.5 mm slices was used. The slices were to begin just inferior to the mandible and end at the superior origin of the temporalis muscle. However, this was not always the case, due in part to varying techniques carried out by different CT technicians. Hence, some muscles, mostly the temporalis, were "cut off" to a small degree at one end. The resulting superior and inferior limits of the CT scans are shown in the CT scoutview seen in Figure 3. Head position was standardized at a 20 degree inferior angulation to the Frankfort plane. The computed tomographic data was then stored on magnetic tape in addition to being displayed as an image on radiographic film. All measurements were

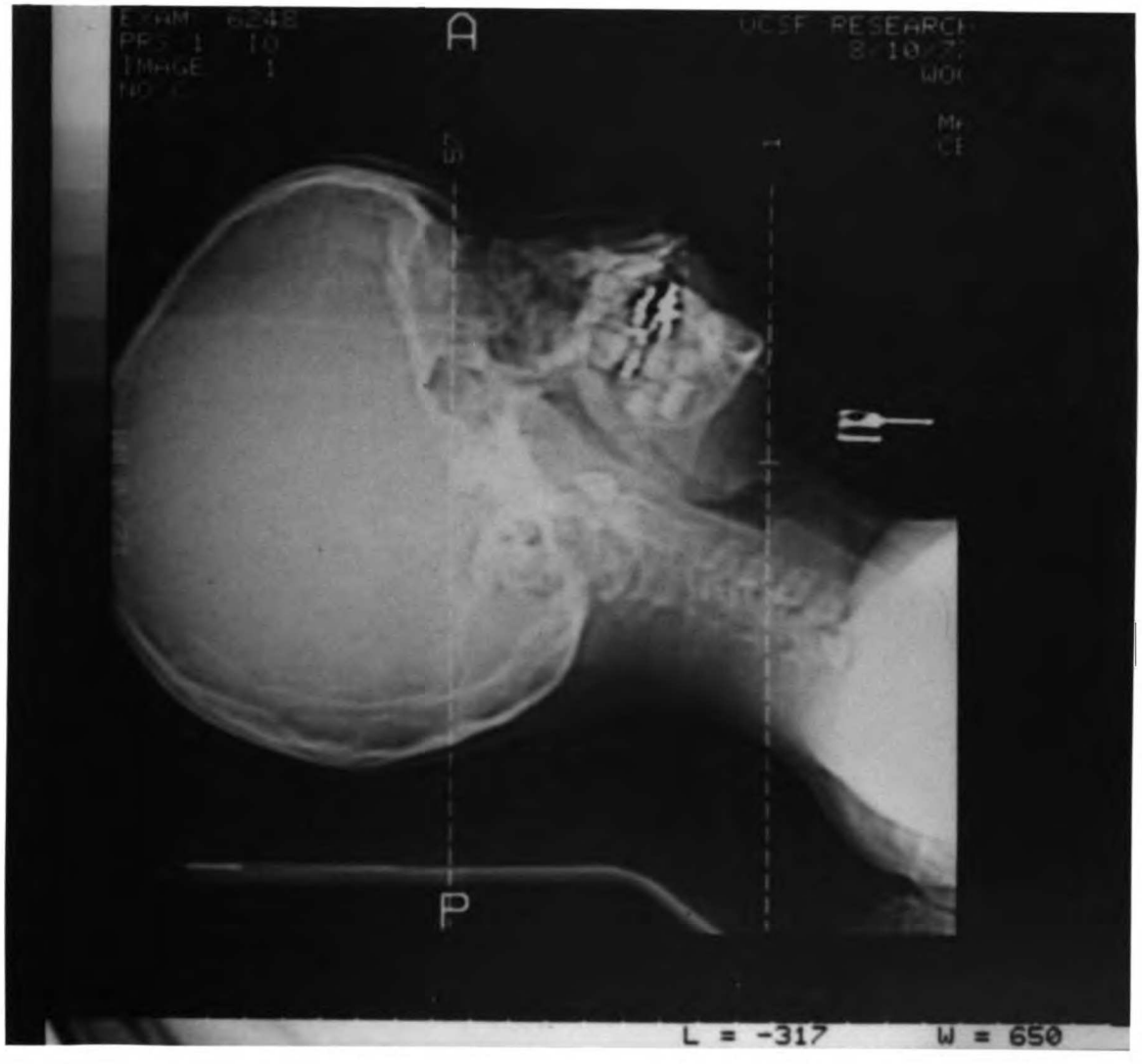


Figure 3: CT scoutview demonstrating the usual superior and inferior limits of CT scans taken on HFM subjects of this study, as indicated by the white, dashed lines.

made on the contiguous images which were viewed on a GE display console. Initially, measurements were made in what will be defined as an "unmatched format". The unmatched format consisted of looking at one scan, either a pre-treatment or post-treatment scan, by itself on the console. This arose primarily due to the fact that one to two years separated the two CT scans taken on the same patient. The unmatched format was also a non-standardized method of data collection. Standardization refers to the CT image viewing parameters mentioned in the Introduction, as well as the forms upon which data was collected and will be discussed in more detail below. Data sets were remeasured, when possible, by a second observer (observer 2) in a "matched format". Matched format refers to a standardized method of data collection and is discussed thoroughly in the next section. The field of view of each image was in the range of 200-250 mm/320 pixels, squared. The resolution, therefore, was about 0.8 mm/pixel.

For each HFM subject, the muscles of mastication were first identified on every slice, if they were indeed present at all. Data was collected with the help of the GE program GEDIS which incorporates irregular region-of-interest software. Axial slices were used for all measurements of the muscles. The specific measurement parameters for each of the four muscles being studied were as follows.

a. Muscle Length

Length of each muscle was determined by identifying the most inferior and most superior slice in which that muscle could positively be identified. The number of slices between these two images was calculated and multiplied by their thickness to give the muscle's length.

b. Muscle Volume

Total volume of each muscle was measured by first identifying the same muscle on every axial slice in which it appeared. The outline of the muscle was then traced and the area was calculated for that slice by the region-of-interest software program. These area values were then multiplied by their respective slice thicknesses and the total muscle volume was determined by adding all the individual slice volumes.

c. Maximum Cross-Sectional Area (MCA) of Muscle

The cross-sectional area of each muscle was measured in the region of its greatest girth and was designated the MCA for maximum cross-sectional area. MCA was operationally defined by examining the cross-sectional areas of all the axial slices and taking the mean of the largest 10% which were also contiguous, independent of the location at which they were found. The importance of this measurement is that cross-sectional area of skeletal muscle has been shown to be proportional to the number of fibers and the strength of the muscle (Knuttgen, 1976, Weijs and Hillen, 1985).

d. Muscle Density

Likewise, the density of each muscle was obtained by first identifying the muscle on each scan it appeared on unless the muscle was somewhat obscured by metal artifacts such as dental restorations (a technical problem, inherent to CT technology). Its boundaries were then traced on the monitor. The software program calculated the average CT number and the standard deviation of that average for all the voxels contained within the outlined region. CT numbers are directly proportional to soft tissue densities and thus were converted to muscle density values (g/cm^3) in this study. For reference,

adipose tissue has a density of 0.9 g/cm^3 and normal muscle is about 1.05 to 1.07 g/cm^3 (Cann, personal communication). The overall density of each muscle was then calculated by averaging the values recorded from all the slices in which the muscle appeared, weighted by each slice's respective cross-sectional area. These QCT measurements thus allow one to calculate the per cent changes in muscle tissue and adipose tissue within a muscle during the testing period. As discussed in the Introduction, QCT calibration data needed to be examined to see if it was necessary to use a QCT correction factor for CT machine-related instability errors. This turned out not to be the case, as data on this showed that the errors were insignificant (Fike et al., 1988).

It should be pointed out that the orientation of the lateral pterygoid muscle relative to the axial scans was different than that of the other three muscles. Despite this, its measurements were made from axial slices exactly like the other muscles. Measurements made on coronal slices which can be constructed by the computer were considered; however, this was not pursued when the results revealed that this would not add useful information since this muscle was frequently absent on the affected side.

2. Matched Format and Standardizations

As discussed in the CT protocol section, each subject's pre-treatment and post-treatment CT scans were originally examined and measured separately in what was called the unmatched format, due in part to the amount of time between the two scans. In an effort to improve the precision of the measurements, standardization of the data collection was developed. This standardization included what is termed a matched format. Under the matched format, pre- and post-treatment scans were matched up slice by slice and then ultimately displayed side by side on the console while being measured. The

Master page referred to in Figure 4 was the document used in creating this matching and an example of it can be found in Appendix A. Images were matched initially based on stable, hard tissue structures, by observer 2 using the hard CT film copies which allowed an overview of all scans. The basis for this criteria was that hard tissue structures are the most easily identified and can be the least affected by time and therapy if not surgically manipulated. Image matching was then verified by consensus of three observers. A new computer file was then created so as to display pre- and post-treatment matched images on a screen simultaneously. This entire matching process usually took three to four weeks per HFM subject.

The matched format provided a number of benefits. By matching the slices, muscles and other structures were more easily and accurately identified. Frequently, a structure was readily discernable on one of a patient's scans and, using it as a reference, was thus visualized on the correspondingly matched scan. Matching also provided for a greater awareness of specific changes from pre-treatment to post-treatment CT scans. Another major benefit was that it allowed reconciliation of variations in the CT imaging protocol that occurred between the same subject's two scans (this is elaborated upon in the Discussion Section).

Standardization in the methodology also applied to CT image viewing parameters. Although the contrast of each image can be varied for better viewing of specific structures by adjusting the CT level and window settings, these values needed to be standardized for the reasons stated in the Introduction. Thus, constant settings of the level at zero and the window at 300 Hounsfield Units for all scans on the same patient and for all observers on the same scans were established to decrease measurement discrepancies due to varying contrast. These values were chosen to emphasize soft tissues in the

images. Likewise, the magnification setting was held constant at 1X between scans and between observers. Another parameter beyond the control of the observer which affects CT size measurements is the defined field of view (DFOV). The DFOV is chosen by the CT technician when the scan is performed. The DFOV indirectly can magnify CT images and the area values reported. This is performed by the computer in the matched format since the computer uses the DFOV in the calculation of a selected area. This was a problem when matched pre- and post-treatment CT scans had different DFOV's. A correction factor was used to correct for these variations. However, it would have been preferable to hold the DFOV constant, which it now is at 21.0 cm; this value allows for the entire width of the head to be viewed optimally.

Lastly, standardization of the data collection was pursued by standardizing the forms that were used (Appendix A). Reference has already been made to the Master page. A second form, the Initial Data Collection Form, was no less important. The organization of these forms helped prevent careless loss of data and thus improved the agreement between observers. Both forms also helped by giving rise to a visual "picture" of each muscle that was measured. This picture included where a muscle began inferiorly and ended superiorly against a common reference scale. Each line on the form was proportional to a CT slice and thus the number of lines a muscle's measurements occupied was proportional to its length. Hence, this "picture" provided visual cues that allowed a muscle to be compared quickly from side to side and from pre-treatment to post-treatment.

With a standardized methodology developed, it was possible for observer 2 to remeasure 2 HFM patients (patients #1 and 3, see Figure 4) under the matched format. The third (HFM patient #2) HFM patient's computer tapes had become unreadable since observer 3 had measured them and so could not be

remeasured. However, a compromise was decided upon to save this valuable data in which this patient's data set was used by rearranging the unmatched data using a matched format (as noted in Figure 4, page 36). Measuring each of the data sets was an arduous procedure which took observer 2, the thesis author, approximately two to three months per data set.

3. Assessment of the Method: Observer Variability

Once the data sets were collected, it was possible to test the hypothesis of Part I. This was done by means of four basic tests, which are diagrammed in Figure 4. The first test was of inter-observer variability where a comparison was made between unmatched and matched data sets. The second test was also one of inter-observer variability, but in this case both sets of data were compiled using the matched format. Differences in individual muscle volumes were examined since this was the broadest measurement category available, encompassing entire muscles and thus less sensitive to a single slice measurement. Paired t-tests and simple linear regression analyses were used to analyze the data. The third test of this section was one of intra-observer variability. Here, observer 1's data sets were put through an unmatched versus a matched format comparison. The fourth test is one in which observer 2 has 2 data sets compared to each other, both in matched format. Again, muscle volumes were used and paired t-tests and regression analyses were employed. It should be noted that when an observer remeasured a CT scan for the second time, at least one month had elapsed since the first measurements had been taken. Furthermore, the observer did not have access to the data of the first observation session when performing the second observation, so as not to be influenced by that information.

4. Assessment of the Method in Relation to Measurement Variability

It was also important to determine if the changes being measured were within or exceeded the range of measurement variability and thus were real. This was possible to carry out in the category of changes in muscle volume by using the standard deviation value from the fourth test of observer variability, discussed in the previous section. Using this standard deviation, the 95%

confidence limits were calculated for observer 2 and compared to the absolute changes in muscle volumes in the patients this observer measured. The objective of this section was to determine if the improvements in QCT standardization resulted in a small enough observer measurement variability to allow detection of small changes in muscle volumes between pre- and post-treatment CT scans.

B. Part II: Control Population

1. Criteria for the Control Population Material

Some clinicians have stated that the contralateral side is normal in HFM patients (Edgerton and Marsh, 1977, Kaban, 1990) and initially, the contralateral side was to serve as the control against which the affected side could be evaluated for changes of the muscles that may have occurred as a result of normal growth and development. It was soon recognized, however, that there were some problems in using this side as the only control. First, there is the distinct possibility that the contralateral side may not have developed normally, but was just not affected to the same degree as the other side. This had not previously been looked at quantitatively. Second, it is quite likely that the surgical treatment of HFM has an effect on the contralateral side as well due to the fact that the entire mandible is being repositioned. For example, the contralateral side may be in a hypertrophic state prior to surgical correction to compensate for the affected side's deficiency. Then, after therapy, the contralateral side's response could potentially be a decrease in mass and density. Therefore, a second control group was desirable. The goal was to obtain a "normal" sample or at least a sample with facial muscles that could be considered normal. By assembling this control sample, the objectives were to gain information about the side to side variation in the muscles of mastication within normal individuals. A second objective was to provide quantitative information about the changes in size and density in these muscles that occur as a result of normal growth and development. To our knowledge, information such as this has not been collected previously.

2. Control Population Selection Criteria and Protocol

Ideally, one would have liked longitudinal data on subjects in the same age range as those seen in the HFM group, using exactly the same protocol. This was not possible since CT is costly and not a totally benign procedure. Thus, normal patients with complete head scans were not available and may not exist. A control sample that was available was a group of patients that had undergone CT scans on a hospital neuro-CT scanner at UCSF usually as a result of a history of intracranial pathology. Their scans happened to use a very similar scanning protocol to this study's protocol and covered a portion of the head that was in our region-of-interest. A total of 23 subjects were ultimately selected, based on similar scanning protocols and, as nearly as could be ascertained, had normal muscles of mastication without pathology. The age range was also restricted, resulting in a range of ages from 6 to 23. These values were chosen so as to cover the ages seen in the HFM group.

An additional criterion for control subject selection was the availability in the subject's CT scan of three representative axial slices. These representative slices were defined as CT slices that could be easily located at the same anatomical locations in every control patient. This, of course, necessitated that head position in each of the scans was consistent; this consistency was judged using CT scoutviews and individual images. These representative slices, in turn, were used to obtain a sampling of data on each of the four bilateral muscles of mastication in the following manner. Representative axial slice number 1, the most inferior of the three slices, was defined as that CT image which included the foramen magnum; furthermore, the image plane had to connect the foramen magnum and the most inferior portion of the anterior attachment of the zygomatic arch to the zygoma. From this slice, the cross-sectional areas and densities of the masseter and medial

pterygoid muscles were obtained. Part of the rationale involved in selecting this anatomical location was ease of identification and the fact that most neuro-CT scans do not proceed any farther inferiorly. Representative slice number 1 cuts through a large part of the belly of the masseter, however, it is usually the most superior slice of the medial pterygoid. Representative slice number 2 was defined as that CT image immediately inferior to the CT image which displays the most inferior portion of the articular eminence. From this slice, the areas and the densities of the lateral pterygoid muscles were measured. This location was chosen because of the ease in which the radiopaque articular eminence could be identified and because the slice cuts through a large portion of the belly of the lateral pterygoids. Representative slice number 3 was defined as the most superior CT image which contained an intact zygomatic arch from anterior to posterior. Temporalis measurements were obtained from this slice, also chosen for its ease of identification and transection of a large portion of the temporalis' belly. It should be noted that if the patient had tilted his/her head from side to side slightly producing asymmetric images, the representative slices might be selected at slightly different levels on the left and right to fit the criteria stated above.

Measurements on all four pairs of masticatory muscles may not have been obtained on every control patient, depending on the superior/inferior extent of their CT scans. Density measurements were not recorded if the patient had been injected with contrast media prior to their scan, as was the case in three of the controls. The cross-sectional area measurments made on these representative slices were termed representative cross-sectional areas or RCA. The densities were converted from CT numbers (RCT) into density values and termed RepDen for representative density. Twenty-two of the 23 control patients underwent this data collection scheme, thus yielding cross-

sectional data in the categories of RCA and RepDen. The corresponding representative slices were located in each of the three HFM patients using the above criteria as applied solely to the contralateral side. This resulted in RCA and RepDen values (if the muscles were large enough to be present, especially true on the affected side) for the HFM subjects which were compared to the control group's values.

It was possible to obtain a complete head and neck CT scan on 1 control subject, a seven year old patient with a history of pneumonia and chronic airway obstruction. Contrast media was used so the density values were invalid. A second problem with this patient was his history of airway obstruction, which is known to influence development and cause what some call "long face syndrome". Thus, this patient's muscles might have been longer than average. However, it was the only data obtainable with respect to somewhat normal muscle volumes and lengths and was used for comparisons in these categories to the HFM subjects.

3. Side to Side Variation within the Control Population

This was tested using the control representative slices. Cross-sectional area side to side variation was the best parameter of size that could be achieved due to the constraints discussed in the previous section. This was carried out by subtracting the RCA of the left side from the RCA of the right side for each of the four muscles of mastication and calculating the means and standard deviations of these differences. The left side was subtracted from the right side instead of taking the absolute difference between the two sides. This was so as not to make the assumption that control subjects are inherently asymmetric, which one would be making if the smaller side was always subtracted from the larger side. The standard deviations were used as

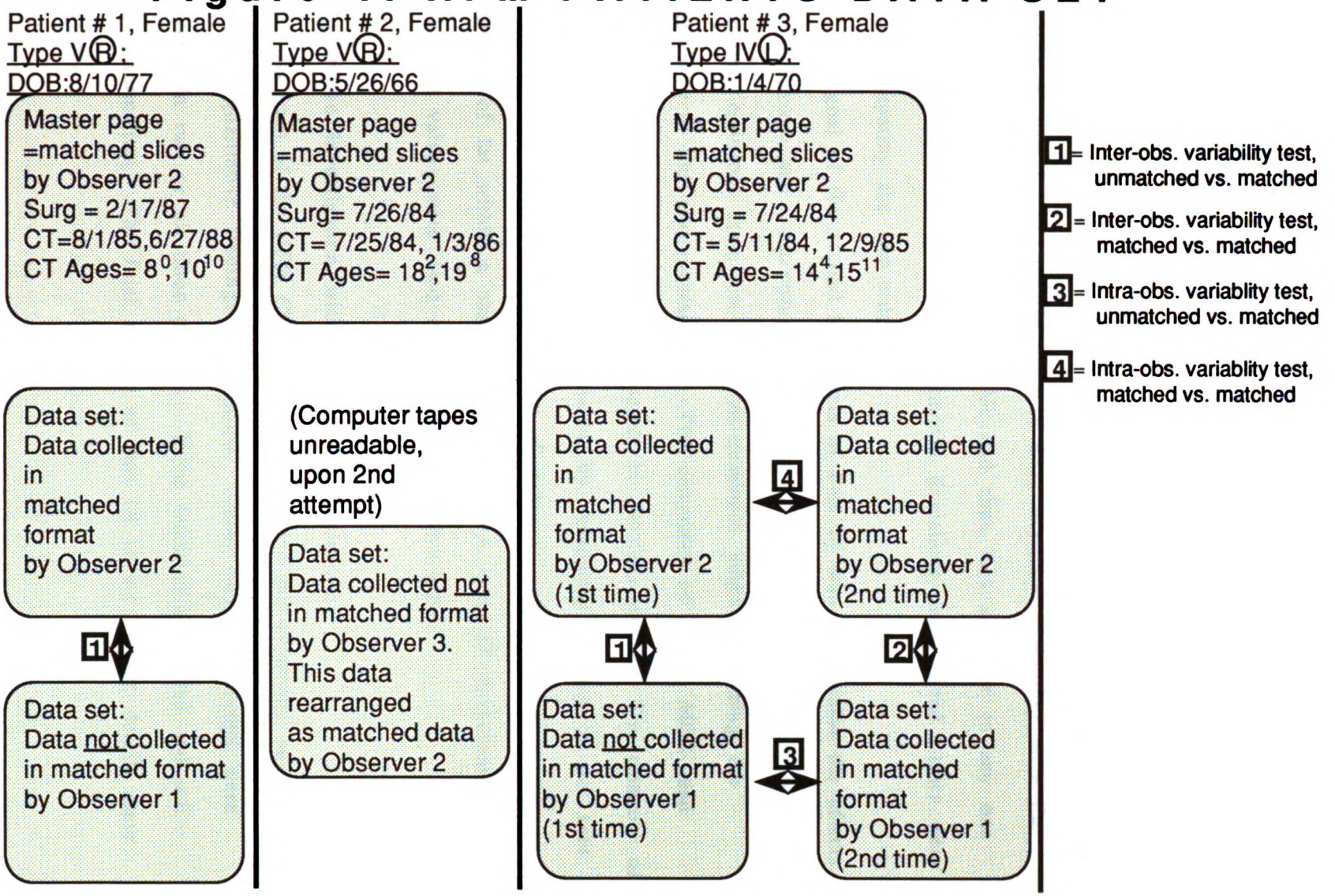
indicators of the amount of variation between the right and left RCA's. Likewise, density side to side variation in controls was addressed using the differences between the right and left RepDen values. Here again standard deviations were used to indicate the degree of variation in each of the muscles of mastication. The hypothesis of Part II was evaluated using these RCA and RepDen standard deviations along with their corresponding means.

C. Part III: HFM Population

1. HFM Population Selection

Patients with HFM are seen regularly at the UCSF Center for Craniofacial Anomalies. Since the incidence of HFM is small, large numbers of these patients are difficult to obtain and only at such centers can any appreciable number of them be found. Type IV and V patients (Table 1) are the second and third most frequently found categories, respectively. As mentioned previously, CT scans using a GE CT 9800 body scanner at the UCSF Research CT Scanner Facility were obtained on these patients just before mandibular surgery (pre-treatment) and approximately twelve to eighteen months after mandibular repositioning (post-treatment) to allow for healing and subsequent function. At the outset of this study, six HFM patients of these types had undergone pre- and post-treatment scans and were thought to be suitable for this study. However, three of these had to be excluded due to incorrect classification of one, atypical surgical procedure in another, and CT records on the third patient's computer tape had become unreadable and back-up tapes could not be located. Thus, the resultant HFM patient sample for this study was three, two Type V and one Type IV (Figure 4).

Figure 4: HFM PATIENTS DATA SET



2. Quantitative Investigations Within the HFM Population

As stated in the Purpose, one goal of this paper was to quantify, for the first time, a portion of the soft tissue asymmetry seen in HFM. The first hypothesis of Part III addressed this issue by comparing the pre-treatment muscle volumes of the affected side to the contralateral (abbreviated contra.) side. As in Part I, only muscle volumes were examined since they were the broadest measurement category available and thus less sensitive to any one single slice measurement. Means, standard deviations, and paired t-tests were used in comparing the two sides.

The goal of the second hypothesis of Part III was to examine the effects of HFM therapy and determine if significant changes in the muscles of mastication occurred from pre- to post-treatment when comparing the affected side to the contralateral side. The categories of length, MCA, and volume were evaluated, looking at changes in absolute values. The category of density, on the other hand, was evaluated by comparing changes in per cent density, so one could speak specifically of increases and decreases in the percentages of fat within a muscle. Again, means, standard deviations, and paired t-test values were used in comparing the two sides.

3. Qualitative Investigation within the HFM Population

As each of the HFM CT scans were measured and examined, new, interesting, qualitative findings were noted. Some similar findings were discovered in two or all three patients. These included both hard and soft tissue abnormalites. The final section of Part III in the Results details these findings.

D. Part IV: HFM Subjects vs. Control Subjects

1. Similarity of Pre-Treatment HFM Contralateral Muscles to Controls

As noted in Part II, the HFM contralateral side has been described as normal (Edgerton and March, 1977, Kaban, 1990). However, data to support this position regarding the contralateral muscles of mastication has been lacking. Three separate analyses in this thesis were employed to address this issue and test the first hypothesis of Part IV. The first analysis was one of size, specifically muscle volume and length. This was possible by using the data from the one complete head scan on the previously mentioned seven year old control patient. It was decided to select one muscle, the masseter (for reasons described in detail below), and compare the control patient's right sided (picked arbitrarily) volume and length to each of the individual, pre-treatment, contralateral masseter muscles of the three HFM subjects. The format of the resultant graph consisted of plotting the volume calculated for each of the individual CT slices of each muscle, against the inferior to superior length of that muscle as determined from CT slice level. This created a graph which would be analogous to dissecting out the patient's masseter muscle, lying it on the table and labelling the inferior end zero millimeters. The "hump" of each graphed muscle corresponds to the belly of the muscle. Thus, a visual "picture" of each of the muscles was created, lining them up at a common origin (example of graph shown in Figure 12a). Similar graphs were created for the post-treatment contralateral and pre- and post-treatment affected sides; these data will be referred to in section three of Part IV. The advantages of this type of graph is that one can quickly compare the lengths and volumes of the muscles graphed upon it. The masseter muscle was chosen for this analysis for the following reasons. One, the temporalis muscle in all of the data sets had been cut-off superiorly during the CT scans, so its total length

and volume could not be represented. Two, the medial and lateral pterygoid muscles of the HFM affected side were usually missing or so small that there was very little to compare with this type of analysis. Also, very small muscles are more difficult to identify and measure accurately using CT. Thus, because its data were usually complete and it was fairly easy to identify and measure, the masseter muscle was chosen. It should be noted that the masseter muscle on the contralateral side in HFM patient number 3, had a small portion cut-off inferiorly in both the pre-treatment and post-treatment scans by the CT technician who performed the scans. This variation was accounted for during data collection through the use of the matched format method, so that equal data were lost between the two scans. This is also represented in the graphs of this first analysis in that the lines corresponding to patient #3's contralateral masseter muscle, do not originate at the graph's origin. The first analysis, therefore, tests the first hypothesis of Part IV with respect to muscle volume and length.

The second analysis was also one of muscle size, that is, cross-sectional area. Four graphs were created, one for each of the muscles of mastication, plotting the RCA values of the 22 control patients against their ages and then constructing a linear regression with the 95% confidence limits indicated. The regression represents changes in RCA as a result of normal growth and development, based on cross-sectional data. Upon these regression analyses, the bilateral individual RCA data points for each of the three HFM patients, pre- and post-treatment, were plotted. The reader is reminded, as explained in Part II, section 2 of the Methods, that some HFM muscles may be absent on their defined representative data slice and thus not have an RCA value. The method described above allowed for RCA comparisons to be made between the controls and the HFM subjects before and after treatment, as well as the

amount and direction of change in RCA that HFM therapy produced versus normal growth. Examination of the HFM pre-treatment, contralateral side data on each of these four RCA graphs permitted the testing of Hypothesis 1 of Part IV with respect to cross-sectional area.

The third analysis was one of density and was initially performed in a similar manner to the regression analysis of the last paragraph. However, regression analyses of the four muscles of mastication on control RepDen values versus age showed that age had no effect on density (data not shown). Therefore, four graphs, one for each of the muscles of mastication, which displayed the control group's mean RepDen +/- two standard deviations and the bilateral individual RepDen data points pre- and post-treatment for each of the HFM patients were created. HFM values which fell outside two control standard deviations (i.e. approximately 95% of a normal population) were considered to be significant. Thus, the similarity of pre-treatment HFM contralateral muscle density to a control population was ascertained by examining the appropriate muscle data points on each of the four RepDen graphs.

2. Side to Side Variation Before and After HFM Therapy

The second hypothesis of Part IV states that the side to side variation in size and density of the masticatory muscles of pre-treatment HFM subjects is greater than that of controls. To test this hypothesis, HFM RCA and RepDen pre-treatment values were graphed against the control data gathered in Part II. Since there were only three HFM patients, it was felt that this data would be more infomative if their RCA and RepDen values were not averaged, but instead each of the individual data points for each patient was plotted. A significant difference was defined as any HFM value that was greater than two standard deviations from the control mean. As usual, a data point was not

plotted if the HFM representative slice did not contain that particular muscle, thus signifying that the muscle was absent from this normal location. In addition, an HFM RepDen value was not used, as always, if it had been affected by a nearby metal artifact, as discussed in the Introduction.

The third hypothesis of Part IV states that HFM side to side variation decreases as a result of the treatment received, approaching the control sample values. To this end, post-treatment HFM RCA and RepDen side to side differences were added to the appropriate graphs described in the previous paragraph. These post-treatment values were compared to the control values, in relation to the pre-treatment points to test if therapy had indeed decreased the amount of HFM side to side variation.

3. Comparison of Muscle Size and Density Before and After HFM Therapy

The fourth and final hypothesis of Part IV states that HFM therapy will increase the size and density of the affected, hypotrophic muscles of HFM, such that these values will become closer to the values seen in controls. This was carried out using the same three analyses discussed in section 1 of Part IV.

As before, the first analysis employed data from the one complete head scan control patient. This information provided a background reference against which changes in affected HFM muscle volume and length could be judged. Again, only the masseter muscle was examined in this first analysis and the data was graphed as described in Part IV, section 1.

The second analysis, pertains to cross-sectional area. Pre- and post-treatment HFM RCA values of both sides were recorded against control data on the same graph (one graph for each masticatory muscle) as previously indicated. This was to allow for greater understanding of the relationships

involved among all the variables. Thus, the effects of HFM therapy and growth on a representative cross-sectional area of muscle were evaluated.

Likewise, the third analysis is exactly as described in section 1. Here, changes in muscle density as a function of treatment were tested. This was performed using the control and HFM RepDen values. Thus, by completing this third analysis, changes in density in hypotrophic muscles during HFM therapy were ascertained and the final portion of the fourth hypothesis of Part IV was addressed.

RESULTS

A. Part I: Standardization of Methods

1. Assessment of the Method: Observer Variability

Hypothesis 1: Inter- and intra-observer QCT measurement variability can be decreased through standardization of QCT technology.

Figure 5 contains simple linear regression analyses representing the variability seen between or within observers in measuring the volumes of the same HFM muscles using QCT. Also reported are the standard deviations between or within the observers when the data was subjected to a paired t-test.

Figure 5a is the results from the inter-observer variability test (see Figure 4) which compared data collected in an unmatched format to measurements taken in the matched format described in the Methods. If the two observers had been in complete agreement when measuring the same muscle volumes, then the slope of the regression line would be equal to one. Furthermore, the y-intercept would be zero and the correlation coefficient (R-squared) would be one. Examining these statistics, Figure 5a displays a fairly high degree of variability between the two observers. The standard deviation, as derived from the paired t-test of this data, is also quite large at 1.394 cm^3.

Figure 5b is also representative of a test of inter-observer variability, however, this time matched formats were employed during each of the observations (see Figure 4). The regression statistics reveal a decrease in variability as compared to Figure 5a. Furthermore, the paired t-test standard deviation has been reduced to 0.375 cm^3.

The third test of this section was an intra-observer variability test, an unmatched versus a matched format comparison involving observer 1

(see Figure 4). The results of this test (not shown graphically) revealed a high degree of variability; the standard deviation being equal to 1.720 cm^3.

The fourth test was also one of intra-observer variability. However, both data set collections were carried out under matched format conditions by observer 2 (Figure 4). The results are shown in Figure 5c. Here, the regression statistics show the smallest variability of all four tests of this section, with the standard deviation value also being quite low at 0.290 cm^3.

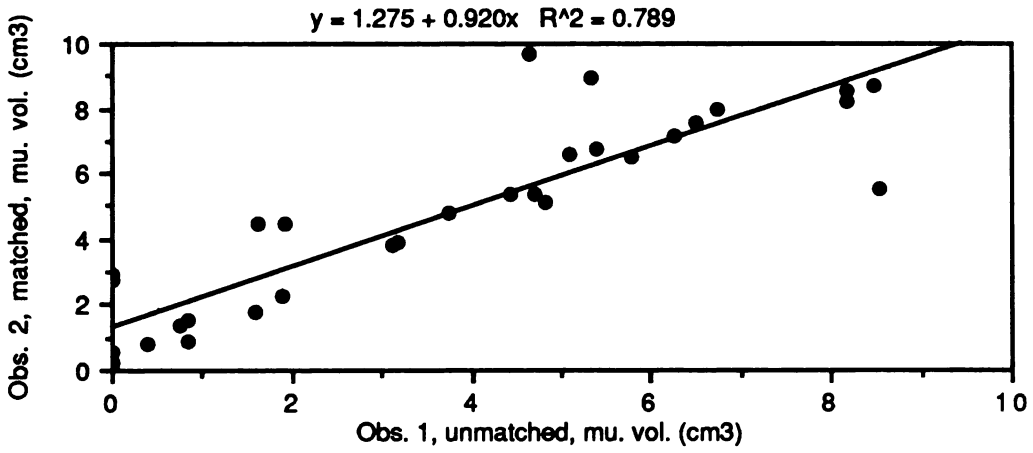


Figure 5a: Inter-observer variability, unmatched vs matched formats; Paired t-test standard deviation = 1.394 cm3

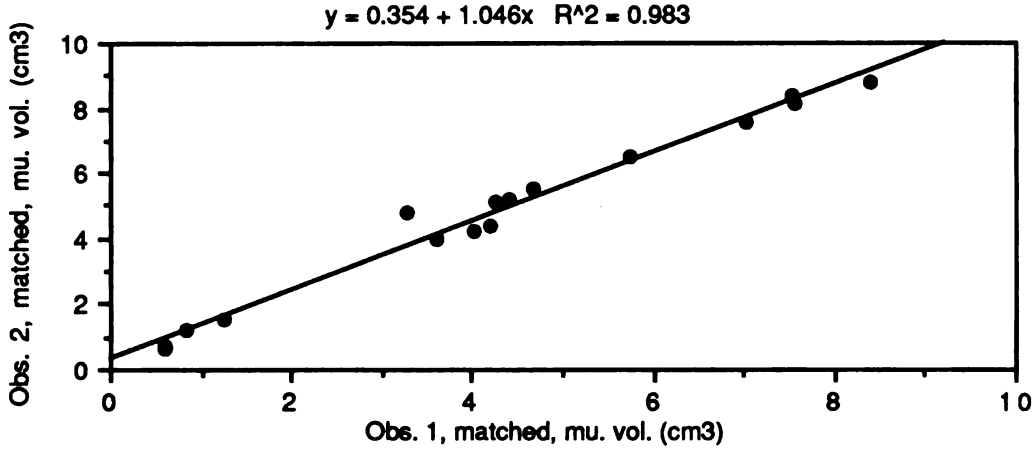


Figure 5b: Inter-observer variability, matched vs matched formats; Paired t-test standard deviation = 0.375 cm3

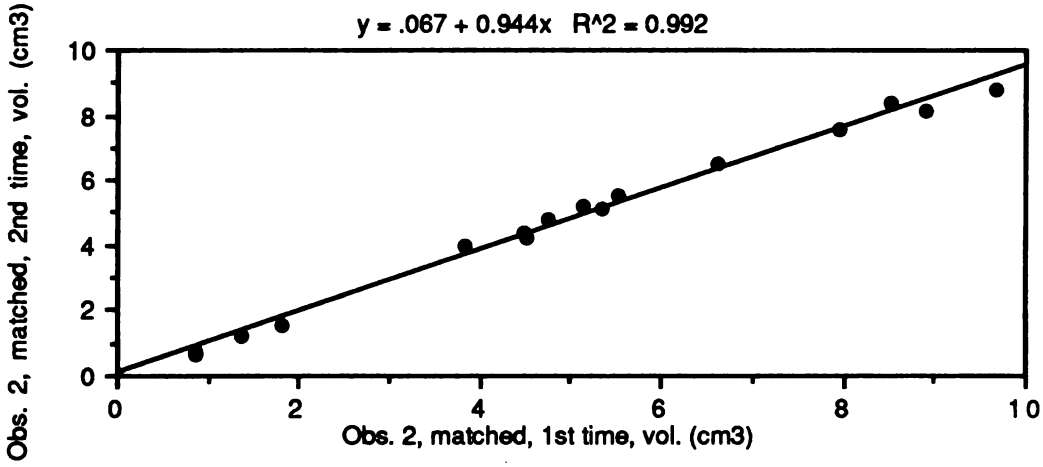


Figure 5c: Intra-observer variability, matched vs matched formats; Paired t-test standard deviation = 0.290 cm3

2. Assessment of the Method in Relation to Measurement Variability

The results of the previous section show that standardization of QCT technology can decrease observer measurement variability. It is equally important to determine if this improvement has produced a measurement tool which is able to detect small differences between two time points. To carry out this objective, the range of measurement variability was calculated for the most improved observer (observer 2) under the most improved observation conditions (standardized, matched format). This range, set at 95%, was calculated using the standard deviation value documented in the fourth test in the previous section of intra-observer variability. Recall that this variability was in regards to measurements of muscle volume, the broadest category that this study examined. The resultant 95% confidence limits were compared to changes in muscle volume during HFM therapy as measured by observer 2 and the results are reported in Table 2. Changes in muscle volume which exceeded the 95% confidence limits are noted, thus, indicating that some of the changes measured were real and not a result of measurement variability. Specifically, the contralateral lateral pterygoid muscle of HFM patient #1 and the contralateral medial pterygoid muscle of HFM patient #3 displayed an increase in muscle volume during their HFM treatment which could not be explained by observer measurement variability.

Table 2: (Post-Tx) - (Pre-Tx) muscle volumes (cm3) vs. measurement variability.

	MASSETER		MEDIAL PTERYGOID		LATERAL PTERYGOID		TEMPORALIS	
	Affected	Contra	Affected	Contra	Affected	Contra	Affected	Contra
Patient #1	-0.22	-0.45	0.30	-0.18	0.00	1.47*	0.69	-0.43
Patient #3	0.35	0.79	-0.3	1.38*	-0.04	0.8	0.14	0.65

* = values > 0.820 cm3, the 95% confidence limits to detect a change between 2 measurements taken by observer #2, based on a standard deviation of 0.290 cm3 (from Figure 5c) between repeated observations by that observer.

B. Part II: Control Population

1. Side to Side Variation within the Control Population

Hypothesis 1: Side to side variation in size and density of the muscles of mastication in a control population is zero.

Size in the control sample was evaluated using the operationally defined RCA values. Figure 6 reports the means +/- one standard deviation for the differences in RCA's between the right and left sides for all four masticatory muscles. All four means were quite close to zero variation and, when analyzed with t-tests, the means were found to not be significantly different from zero.

Density in the control sample was evaluated using the operationally defined RepDen values. Figure 7 shows the means +/- one standard deviation for the difference in RepDen's between the right and left sides for all four muscles of mastication. Again, using t-tests all four means were found to not be significantly different from zero. Thus, Part II's hypothesis can be accepted with respect to both size and density.

Figure 6: RCA Side to Side Variation, Controls

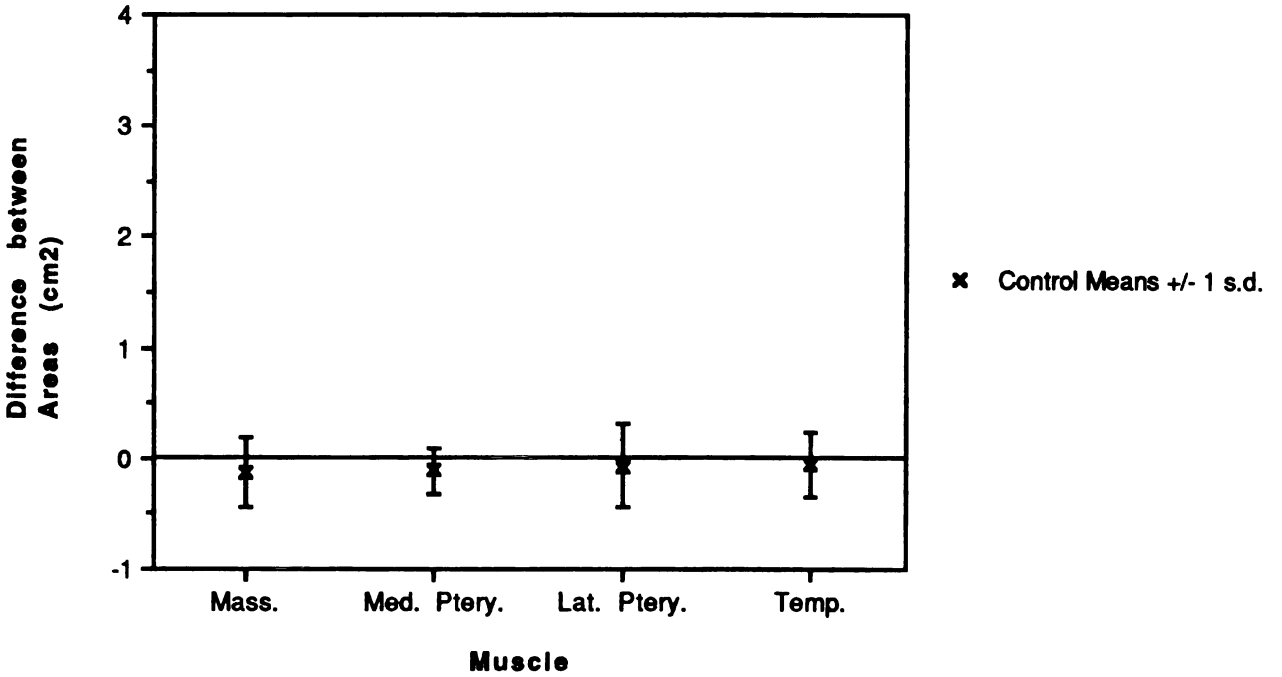
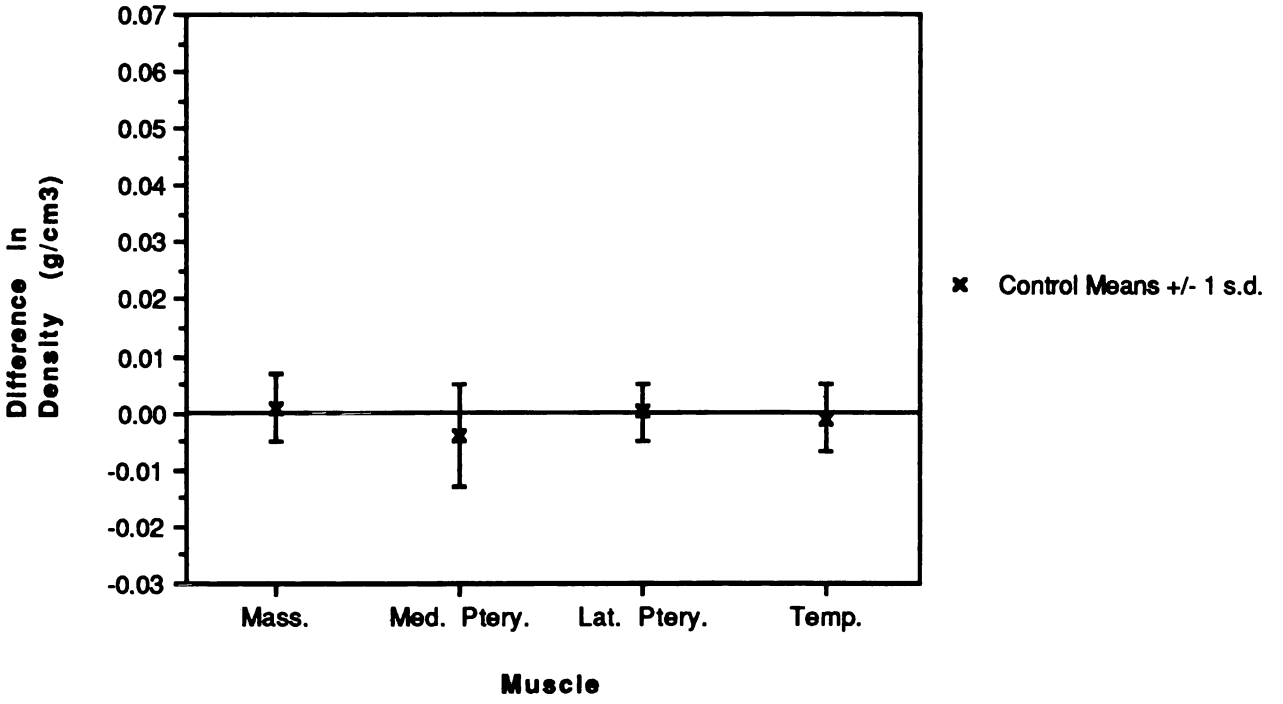


Figure 7: RepDen Side to Side Variation, Controls



C. Part III: HFM Population

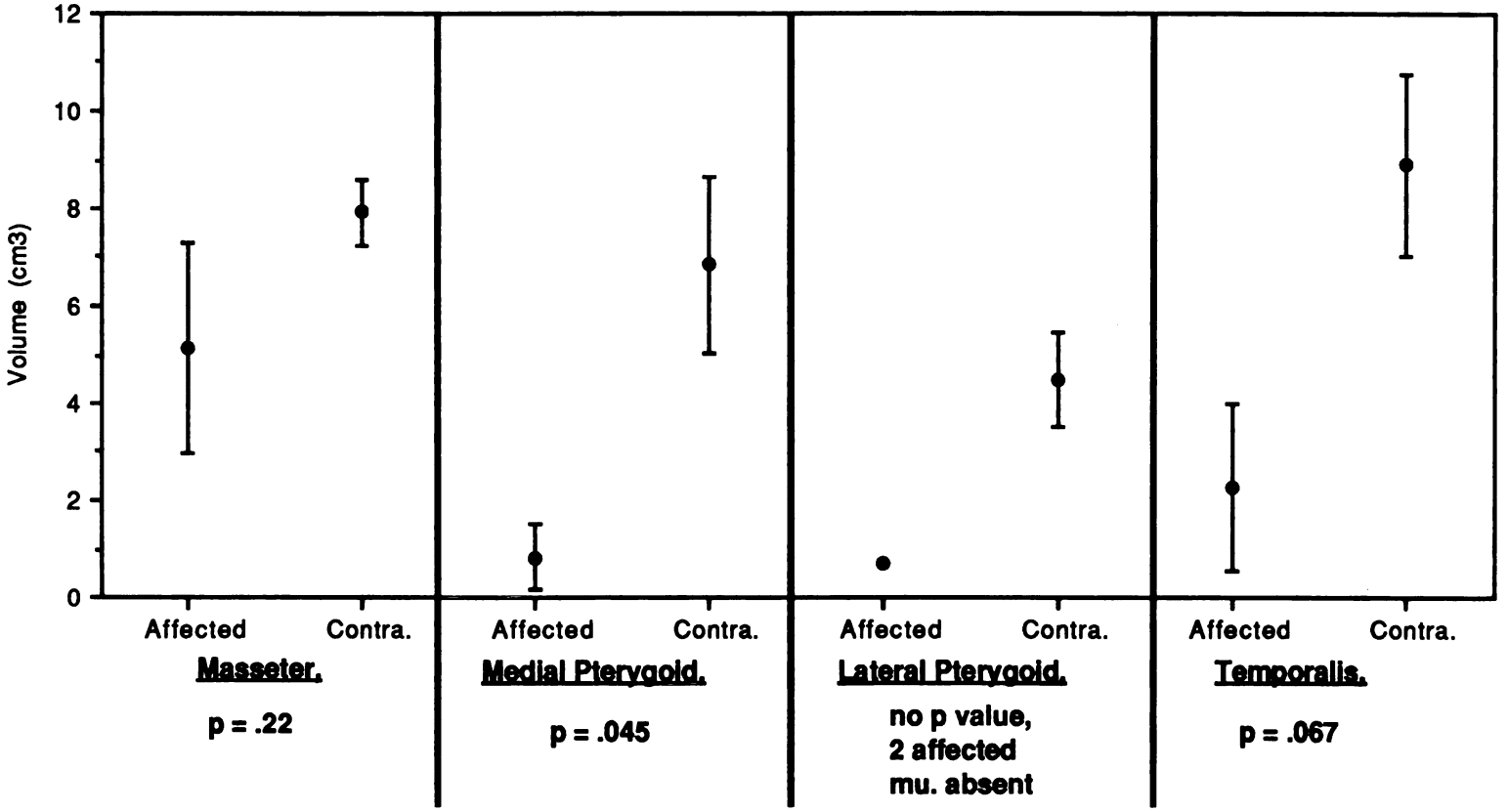
1. Quantitative Findings within the HFM Population

Hypothesis 1: The pre-treatment volumes of each of the muscles of mastication on the affected side in HFM are less than those of the contralateral side.

Pre-treatment muscle volumes for the three HFM subjects of this study were averaged and the resultant means and standard deviation are shown in Figure 8. A paired t-test was performed on each of the masticatory muscle pairs to determine if there were any statistically significant differences between the two sides. By the means, all four affected muscle groups had smaller volumes than their contralateral antimeres. However, the differences in volume between the affected and contralateral masseter muscles were not significant ($p = .22$).

On the other hand, the affected medial pterygoid muscles were significantly smaller than their contralateral counterparts ($p = .045$). In the case of the lateral pterygoids a paired t-test could not be performed; however, significant results existed here as well because in the two HFM Type V subjects the affected side lateral pterygoid was totally absent. Thus, only one affected lateral pterygoid measurement was registered for this HFM sample. The volume of this one affected lateral pterygoid was quite small in comparison to the three contralateral muscles. Finally, the volumes of the affected temporalis muscles were also considered to be significantly less than their contralaterals, $p = .067$. Thus, the hypothesis that the pre-treatment muscle volumes of the affected side are less than those volumes of the muscles of the contralateral side could not be rejected.

Figure 8: HFM Pre-Tx Muscle Volume Comparison, Affected vs Contralateral Side; N=3



Hypothesis 2: Surgical Reconstruction of the mandible and rehabilitation will increase the size and density of affected side muscles as compared to muscles of the contralateral side.

The four graphs of Figure 9 address this hypothesis, one for each muscle of mastication. Each of these graphs was organized in the same fashion to give an overall view of the changes that occurred in the muscle in question during HFM treatment. Thus, changes in the four categories of length, MCA, volume, and per cent density are reported in each of the graphs.

Figure 9a examines changes seen in HFM masseter muscles during treatment. When comparing the affected side to the contralateral side muscles, no significant changes were revealed in size or density. Changes in HFM medial pterygoid muscles are shown in Figure 9b. There were no significant changes to report in the categories of size. However, there was a statistically significant decrease in density of the affected side muscles when compared to their antimeres. The contralateral medial pterygoids, on average, did not change in density during treatment and thus can be used as a standard to describe what occurred on the affected side. Doing so, one finds that the medial pterygoids of the affected side experienced on average a 1.10% decrease in density. This is equivalent to a 6.30% increase (15% pre-tx to 21.3% post-tx) in the amount of adipose tissue in the affected medial pterygoids as calculated from the equations:

$$\rho_{total} = (F_m)(\rho_m) + (F_f)(\rho_f) \quad \text{and} \quad F_m + F_f = 1$$

where ρ_{total} is the average density measured for an entire muscle; F_m and F_f are the fractions of the muscle that are muscle tissue and fat tissue in per cent, respectively; and ρ_m and ρ_f are the densities of pure muscle tissue and pure fat tissue (1.06 and 0.9 g/cm^3), respectively. Solving for the pre- and post-

treatment values of F_f in the first equation algebraically and then finding their diffence yields the change in the muscle's adipose tissue content.

Figure 9c represents the data for the lateral pterygoids. Since two of the three HFM subjects did not have lateral pterygoids on the affected side, it is not possible to compare statistically treatment changes on the affected side to the contralateral side. However, a t-test on the MCA and volume changes observed in the contralateral lateral pteryoids reveal that these increases in size have a less than 10% probability of being the result of random chance. Recall also from Table 2 of Part I that these increases in volume were most likely real and not the result of measurement variability. Thus, it would appear that the contralateral lateral pterygoid muscle increases in size during the course of HFM therapy.

Figure 9d presents the data for changes in the temporalis muscles during HFM treatment. No values are given in the length category since none of the CT scans extended superiorly far enough to show the superior limit of these muscles, as noted in Part I of the Methods section. The matched format process helped ensure that equal amounts of information were lost due to this limitation. However, true length measurements could not be obtained. With respect to the remaining categories, no significant changes in size or density were revealed when the affected side was compared to its contralateral counterpart.

In summation, the size and density of the affected side muscles of HFM did not significantly change when compared to the muscles of the contralateral side over the course of HFM therapy with one notable exception. This exception was a decrease in density of the affected medial pterygoids when compared to their antimeres. Thus, the second hypothesis of Part III is rejected.

Figure 9a: Masseter, HFM Tx Effects, Affected vs Contralateral Side; N=3

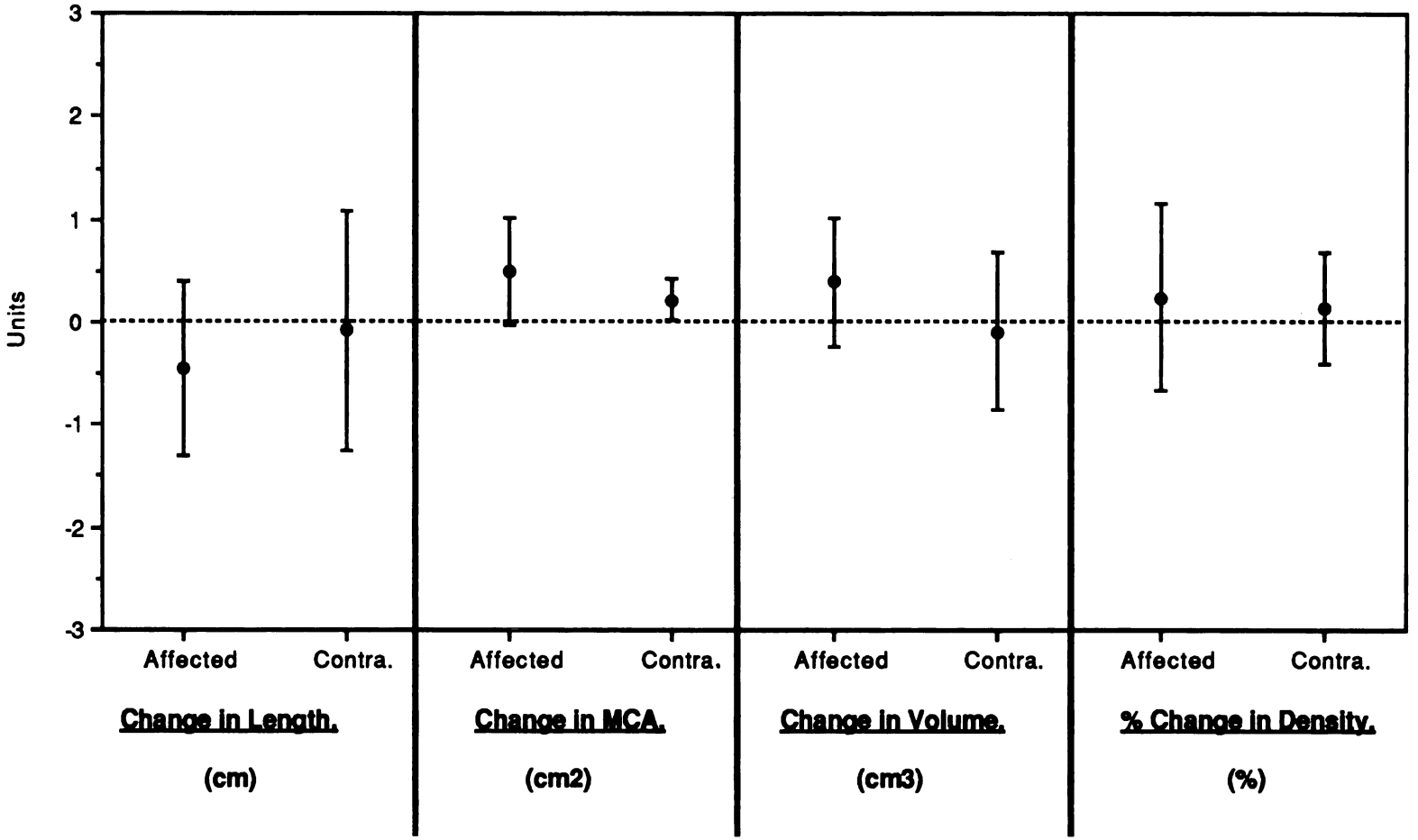


Figure 9b: Medial Pterygoid, HFM Tx Effects, Affected vs Contralateral Side; N=3

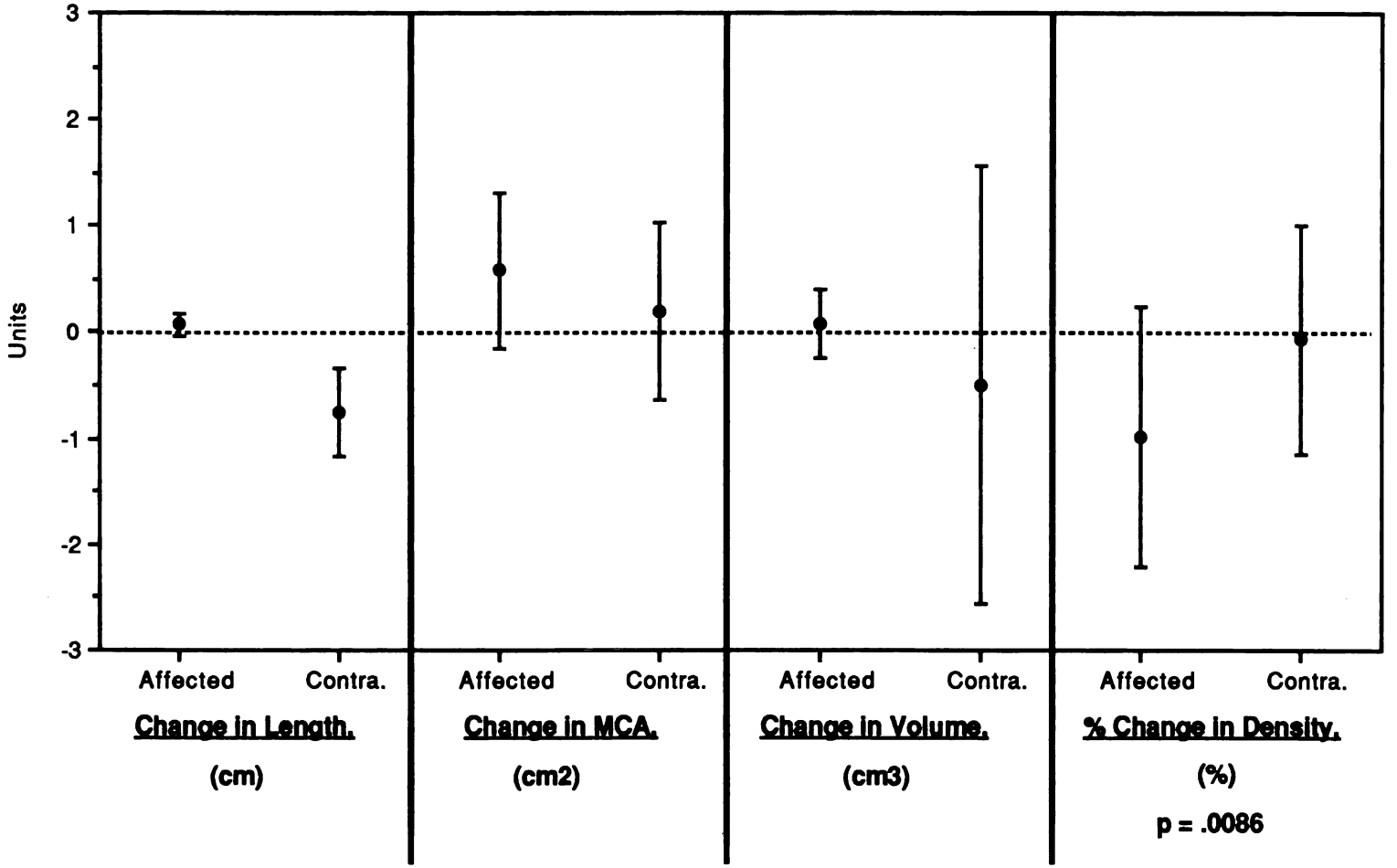


Figure 9c: Lateral Pterygoid, HFM Tx Effects, Affected vs Contralateral Side; N=3

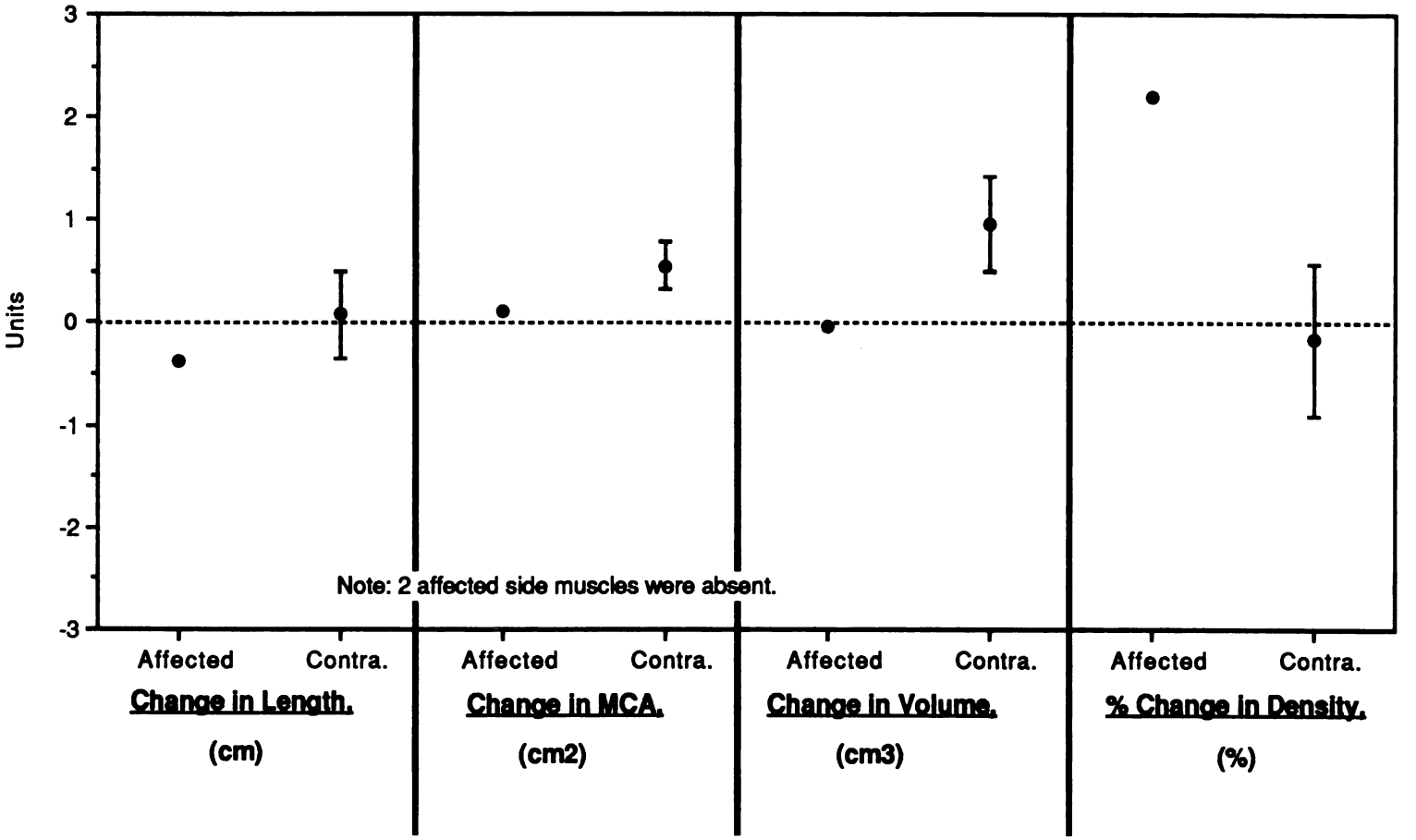
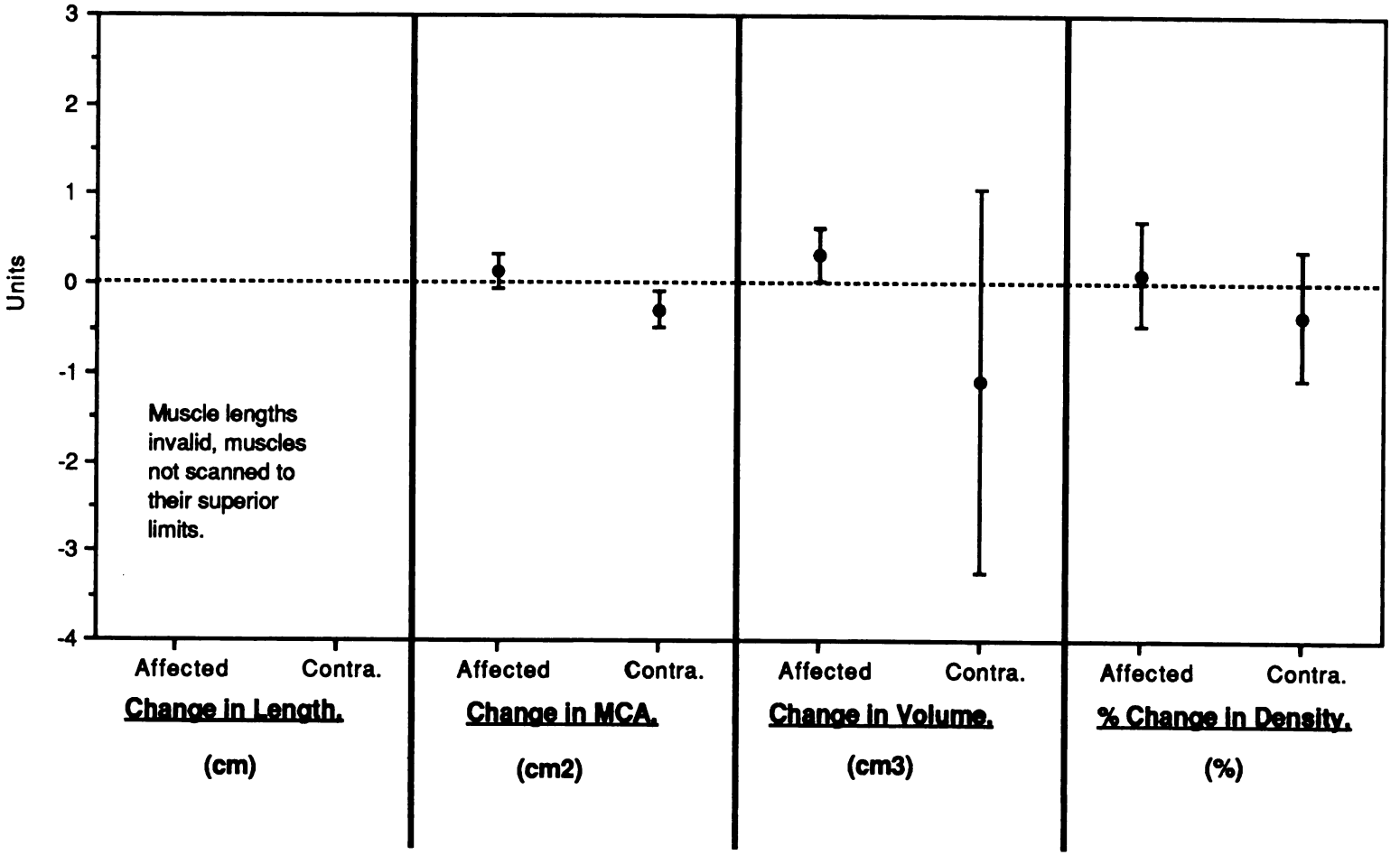


Figure 9d: Temporalis, HFM Tx Effects, Affected vs Contralateral Side; N=3



2. Qualitative Findings within the HFM Population

Some interesting, previously unreported, qualitative findings were discovered during the course of this study. There were a number of soft tissue abnormalities which could be easily discerned using CT. One feature in common to all three HFM subjects in this study was that the affected muscles, when present, usually had normal origins but inserted at more superior positions than seen on the contralateral side and in the controls. This finding is not so surprising considering that the normal place of insertion, the mandible, was deficient on the affected side. Frequently, the affected masticatory muscles appeared to insert into small fibrous bundles of tissue which ultimately blended into the surrounding connective tissue inferiorly. The net results of this were affected muscles, particularly the masseter, medial pterygoid, and temporalis, which were shorter in length and smaller in volume than their contralateral counterparts both prior to and after treatment. As mentioned previously, the affected side lateral pterygoids were completely absent in the two Type V HFM subjects. HFM patient #3, the Type IV patient, did have a lateral pterygoid on the affected side, but it was quite small. It was also fragmented, the muscle really consisting of bundles of muscle fibers interspersed with adipose tissue.

The medial pterygoids on the affected side were also quite diminuitive in the Type V patients, being located only in the region between the pterygoid plates and never attached to the mandible. On the other hand, HFM patient #3's affected medial pterygoid was a little larger than that of the other two subjects and did attach to the mandibular ramus.

There was one notable exception to the usually normal origination of the affected side muscles of mastication. The affected masseters of HFM patients #2 and #3 did not appear to originate on the inferior and deep

surfaces of the zygomatic arch as is normal. Rather, these muscles could be followed well onto the exterior surface of the zygomatic arch.

One other soft tissue abnormality appeared in all three HFM subjects. This was an area of poorly vascularized adipose tissue present in the position of the missing mandibular ramus (see Figure 10). This "black hole" as it appeared on the CT images, had an extremely low density reading, more consistent with that found in abdominal fatty tissue (Cann, personal communication) than that of facial adipose tissue. An interesting note, the CT scans revealed that the surgeon placed the bone graft in all three cases just laterally to this unusual area of adipose tissue. This occurred as he positioned the superior end of the graft against the zygomatic arch in the area of the absent glenoid fossa.

There were two other hard tissue findings concerning the graft placement. All three grafts could be seen wired to the external surface of the most posterior limit of the affected mandible. Furthermore, as each graft was directed towards the absent glenoid fossa, it came in intimate contact with the posterior surface of the affected masseter muscles (see Figure 10). Whether or not an attachment developed between this muscle and the graft can not be concluded from CT evidence alone.

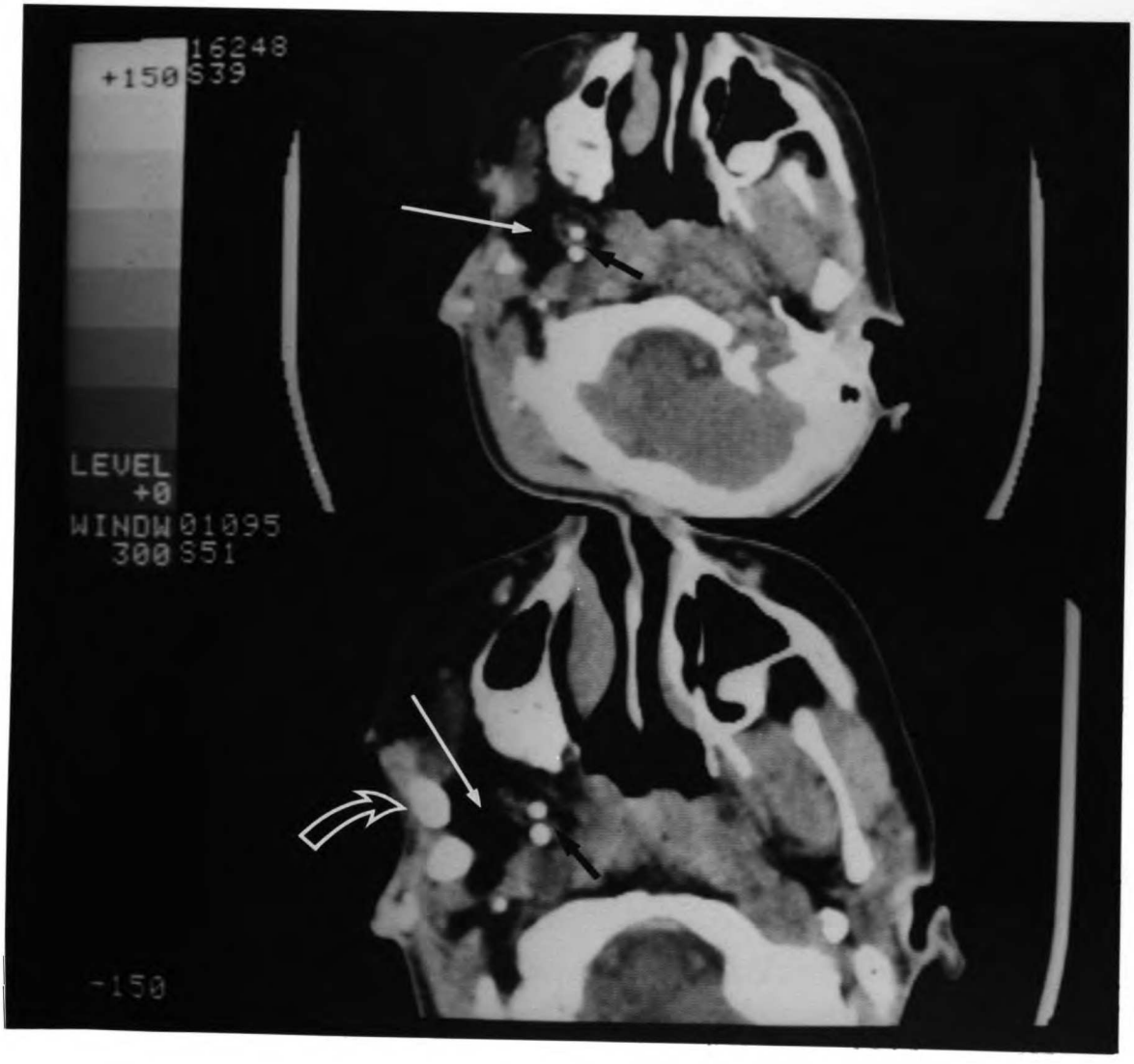


Figure 10: Two axial CT images in matched format of the same patient. These images are from HFM patient #1 at the level of the nasal cavity; pre-tx image is above and post-tx image is below. Solid white arrows indicate an area of poorly vascularized adipose tissue in the area of the missing mandibular ramus. Outlined white arrow points to a costochondral bone graft. The right masseter muscle can be seen just anterior to the graft. Black arrows indicate two unidentified calcified structures (see text).

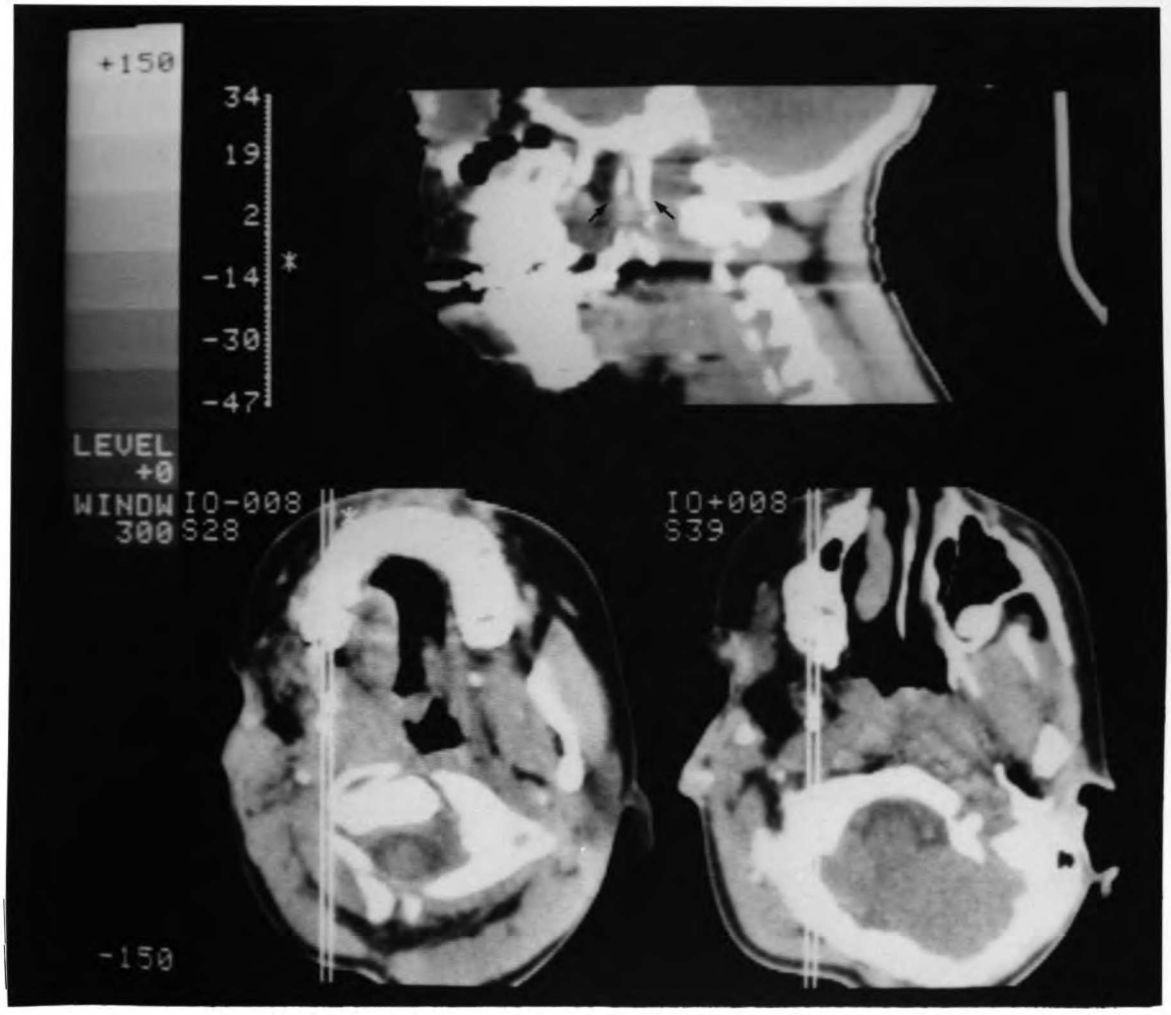


Figure 11: Construction of a para-sagittal CT image from two pre-tx axial CT images. All three images are from HFM patient #1. The lower right image (S39) is the same pre-tx image seen in Figure 10; the lower left image (S28) is from 15 mm. inferior to S39 at the level of the maxilla. The two white parallel lines indicate from where the para-sagittal plane was reconstructed. Note that this plane bisects the two unidentified calcified structures mentioned in the text. The resultant para-sagittal image is seen as the top image and the two small black arrows point out the unidentified calcified structures.

One final qualitative finding of interest seen in HFM patients #1 and #2 was another hard tissue abnormality. Two, transversely round, calcified structures which the investigators were unable to identify were discovered in a region just posterior to the affected side maxilla in the infratemporal fossa (see Figure 10). They were in intimate contact with the fragmented inferior portions of the affected side temporalis muscle. They were oriented with one calcified structure being posterior to the other. Both structures appeared to have some superior to inferior length with some breaks in continuity. A para-sagittal CT view of these structures revealed that they were attached to the cranial base superiorly (see Figure 11). In one post-treatment CT image, one of the structures appeared to have developed a bone marrow space internally. One possible identification for these two structures is calcified ligaments.

D. Part IV: HFM Subjects vs. Control Subjects

1. Similarity of Pre-Treatment HFM Contralateral Muscles to Controls

Hypothesis 1: Prior to HFM therapy, the size and density of the muscles of mastication on the contralateral side in HFM are similar to the muscles of mastication of a control population.

Three separate analyses, described in Part IV, section 1, of the Methods, were used to test this first hypothesis. Results of the first analysis are shown in Figure 12a. This graph plotted the volume of each respective CT slice going from zero millimeters inferiorly, to 60 millimeters superiorly. Slice thicknesses for the one, seven year old, control patient were five millimeters, thus explaining the smaller number of points (represented by the horizontal portions of this patient's line) on the graph. Slice thicknesses for most of the HFM data, recall, were 1.5 mm. Figure 12a demonstrates that the lengths of the contralateral HFM masseters were quite similar to that of the control patient's masseter. However, total volumes, the areas represented under each line graphed, were quite dissimilar. The volume of the control patient's masseter was much greater than each of the HFM contralateral masseters prior to HFM therapy.

The second analysis was also concerned with a category of muscle size, cross-sectional area, by means of the previously defined RCA values. This data is shown in Figure 13a, b, c, and d; one graph being used for each muscle of mastication. Each of these graphs were constructed by first plotting the control RCA values (not shown) as a function of age and then subjecting this data to a simple linear regression analysis, which is represented by the solid line. The 95% confidence limits of these regression lines were then added, as represented by the dashed lines. A quick glance at each of the four graphs of Figure 13 relates that cross-sectional muscle area as measured by RCA in

controls increases with age in a linear fashion. While an increase in size with increasing age was expected, the fact that it was linear may be an incorrect assumption which will be considered in the Discussion Section.

Having constructed Figure 13 in this fashion, the RCA values for each of the possible HFM measurements were overlayed on the graphs as shown. HFM data points which were related to each other through the dimension of time were connected by dotted (contralateral side) and dashed/dotted (affected side) lines. Thus, one can compare HFM RCA values to the background of control values. Furthermore, one can appreciate the amount and direction of change in HFM RCA during HFM therapy in each of the three HFM subjects.

Note the HFM, pre-treatment, contralateral side RCA values of Figure 13 as compared to the normal range of the controls. With respect to the masseter (Figure 13a), the two older HFM patients had contralateral muscles that were smaller than the controls whereas the youngest subject fell within normal limits. Examining the contralateral medial pterygoids (Figure 13b), one found a large amount of variation in the three HFM subjects with respect to normal limits. However, both masseter and medial pterygoid pre-treatment contralateral RCA values displayed an interesting tendency to decrease with age in direct opposition to the control's tendency. Figure 13c shows that the two HFM Type V patients have pre-treatment contralateral lateral pterygoids that fell within the normal range but HFM patient #3 had an RCA below normal. The results for the temporalis muscle (Figure 13d) also appeared variable. Thus, with respect to cross-sectional area as measured by RCA, one would have to conclude that the results are mixed as to whether HFM pre-treatment contralateral muscles are similar to control muscles.

Density is the other half of the statement given in Part IV's first hypothesis and this is addressed in the third analysis of this section. This test was initially carried out in a very similar manner to the RCA test of the second analysis. However, regression analyses comparing masticatory muscle density (as measured by RepDen) versus age showed that age had no effect on density (data not shown). Therefore, the four graphs of Figure 14 compare HFM RepDen values to the control group's mean RepDens +/- two standard deviations. Those HFM values which fell outside two standard deviations were considered to be significantly different from the control population.

Figure 14a shows that the HFM pre-treatment contralateral masseters had densities that fell within the range of normal. This was also the case for the medial pterygoids (Figure 14b), the lateral pterygoids (Figure 14c), and the temporalis muscles (Figure 14d).

Therefore, the results of the first two analyses of this section indicated that the HFM pre-treatment contralateral muscles of mastication were more dissimilar than similar to the masticatory muscles of a control population with respect to size, with the possible exception of muscle length. The third analysis, however, indicated that these same HFM contralateral muscles were quite similar in the category of muscle density to control muscles.

2. Side to Side Variation Before and After HFM Therapy

Hypothesis 2: The side to side variation in size and density of the muscles of mastication of pre-treatment HFM subjects is greater than that of control subjects.

Tests for this hypothesis utilized again the values of RCA and RepDen as measurements of size and density. Figure 15 shows the data for the variable of size. The control data is the same that was presented in Part II, except that the

error bars now represent two standard deviations instead of one. At this time, focus only on the pre-treatment HFM values as compared to the controls. In virtually every case for all four muscles, the HFM subjects displayed side to side variation that exceeded the two standard deviations range of the controls. The two exceptions were in the masseter and medial pterygoid muscles of HFM patient #2, where the differences between right and left RCA's were just at the two standard deviations limit of the controls. As previously stated, muscles which were not seen on a representative slice resulted in no value and thus are missing from the graph in Figure 15.

Figure 16 is the side to side variation test of density, carried out in the same fashion as Figure 15, using RepDen values. Here again, the majority of HFM subjects exhibited more pre-treatment variation than seen in the controls. Exceptions were in the masseter and lateral pterygoid muscles of HFM patient #2 and the temporalis muscle of HFM patient #3. Missing data points, again, signify missing muscles or metal artifact scatter interference.

Thus, hypothesis 2 of Part IV can not be accepted without some hesitation, as interpreted by these results. The majority of the information suggests that there is a greater amount of variation between the two sides in HFM subjects vs. controls, especially with respect to size. A larger HFM sample size might provide greater confirmation of this hypothesis.

Hypothesis 3: The HFM muscles' side to side variation in size and density will decrease as a result of treatment, approaching the control population values.

This hypothesis is also dealt with in Figures 15 and 16 by comparing the HFM post-treatment values with the HFM pre-treatment values, in relationship to the range of the controls. Variation in side to side RCA (Figure 15) appears

to increase in almost every case following HFM therapy. Only temporalis muscles show a small tendency to decrease in variation.

Side to side variation in density, on the other hand (Figure 16), had mixed results. All HFM masseters and medial pterygoids displayed an increase in variation. Lateral pterygoids demonstrated a decrease in density variation between the two sides with post-treatment differences falling within normal limits. Temporalis muscles' variation stayed about the same or slightly decreased.

The majority of the evidence, therefore, points in the direction of an increase in the side to side variation in size and density of HFM muscles after treatment as compared to controls. Thus, Part IV, hypothesis 3 is rejected.

3. Comparison of Muscle Size and Density Before and After HFM Therapy

Hypothesis 4: HFM therapy will increase the size and density of the affected, hypotrophic muscles of HFM, such that these values will become closer to those values seen in controls.

This hypothesis was tested using the same three analyses discussed in section 1 of this Part and shown in Figures 12, 13, and 14. In the first analysis, changes in HFM affected masseter muscle length and volume were compared to the seven year old control patient that was previously mentioned (see Figures 12b and 12c). Pre-treatment, all three HFM affected masseters were shorter and contained much less volume than the control. Another interesting feature was that, although the control patient's graph was a monotonic function, the HFM masseters were bi-modal or tri-modal. Comparing Figure 12b to Figure 12c, one can appreciate the changes that occurred in these masseter muscles over the course of treatment. One result was that the post-treatment affected masseters attained a shape that was now more similar to

that of the control. A second result was that HFM patient #2's masseter increased in length to equal the muscle length seen in the control, without increasing its volume. Hence, this masseter must have been "stretched out" over the course of treatment. For completeness, a graph containing the HFM post-treatment contralateral masseters (Figure 12d) was also included. Small increases in length appear to have occurred in these muscles as well, also probably due to being stretched out (compare to Figure 12a).

The second analysis, as in Part IV, section 1, also dealt with size but in terms of RCA. Results are displayed in Figure 13a, b, c, and d. These four graphs show how RCA values for the four muscles of mastication changed over the course of HFM therapy. By comparing the HFM values to the control range of values, it was possible to ascertain if the HFM RCA's were within normal limits and if the rates of change were greater than, less than, or equal to the rates of change as seen in a cross-sectional control sample. There was really only one consistent finding among any and all of the four graphs of Figure 13 and that was inconsistency. The directions, amounts, and rates of change in RCA for both the HFM affected and contralateral muscles displayed a great amount of variation and inconsistency. There were a number of possible reasons for this and they are explored in the Discussion section.

The third analysis, as before, was of density (Figure 14) through the use of RepDen values. The format of the four graphs of Figure 14 is as described in section 1 of Part IV. As with the RCA values, changes in RepDen values also displayed a great deal of variation. Some affected muscles increased in density, approaching control values, while others decreased. None of the affected muscles' RepDens were greater than the control ranges before or after treatment. Changes in the HFM contralateral muscles were variable as well.

Hence, the evidence to date did not support the concept that HFM therapy, as rendered in this study, resulted in an increase in the size and density of affected muscles such that their values were normalized. At the present time then, the fourth hypothesis of Part IV can not be accepted.

Figure 12a: Masseter, Pre-Tx Contralateral Side, HFM vs Control

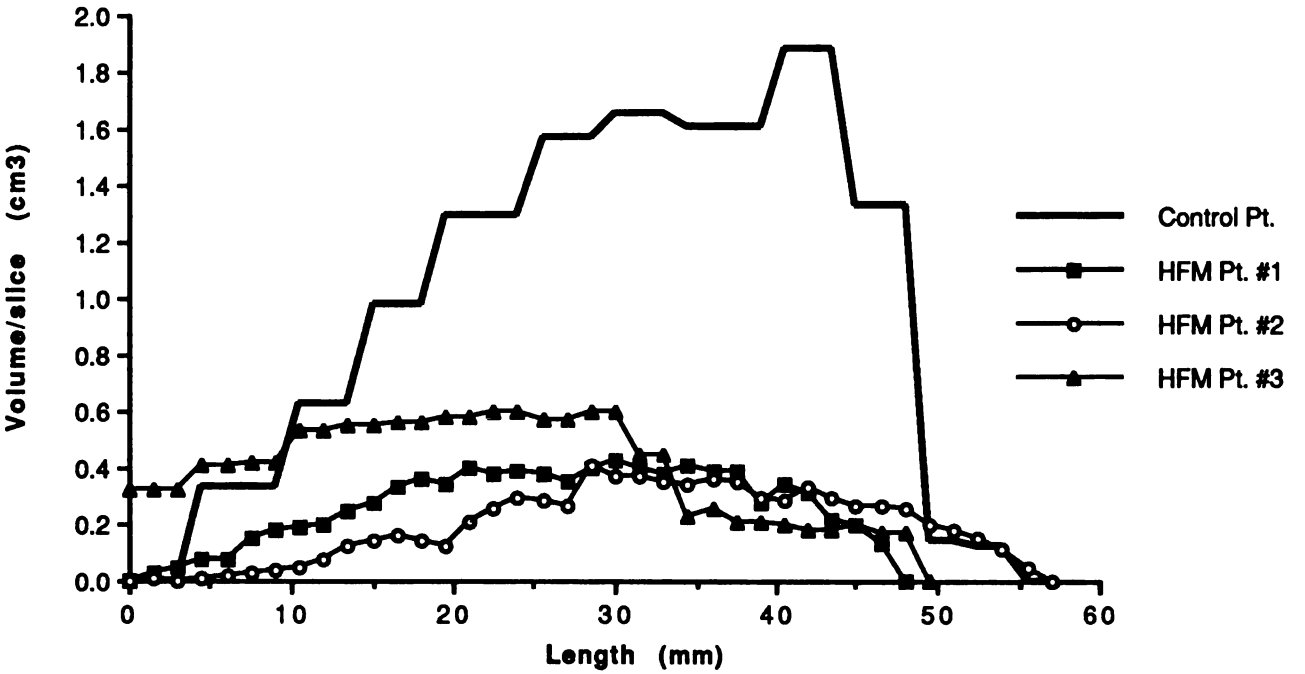


Figure 12b: Masseter, Pre-Tx Affected Side, HFM vs Control

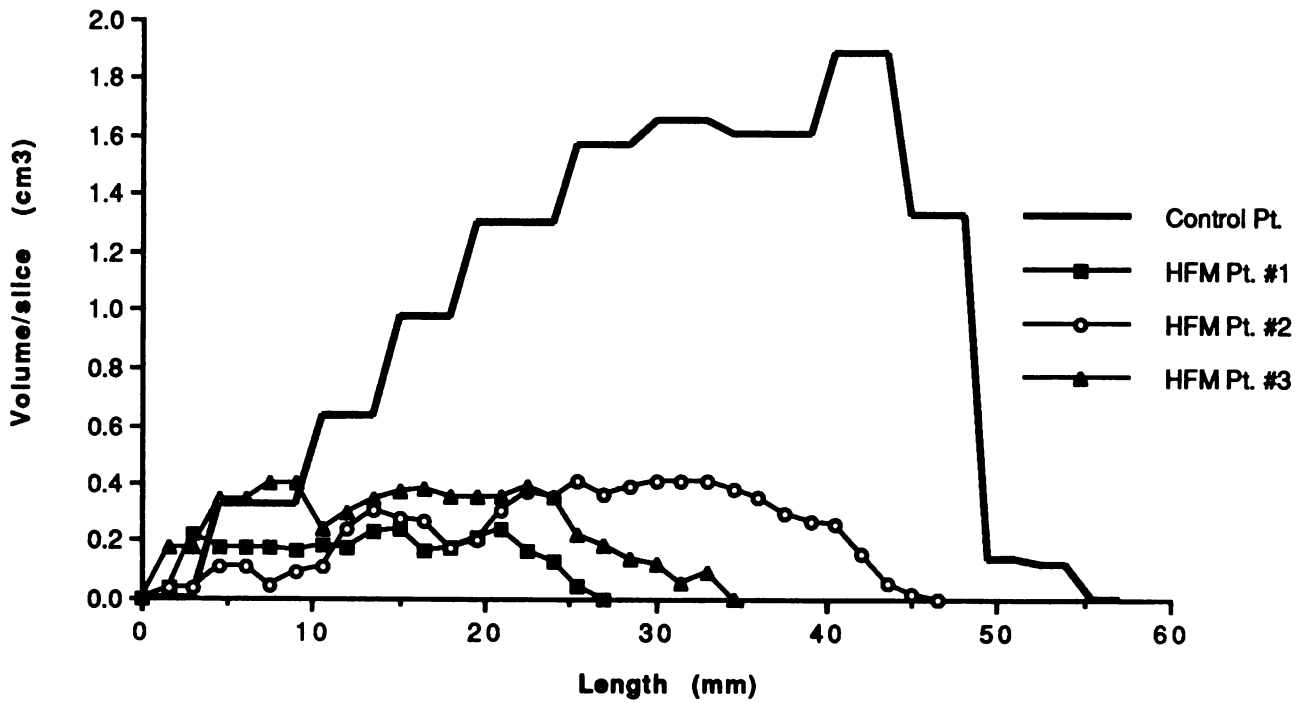


Figure 12c: Masseter, Post-Tx Affected Side, HFM vs Control

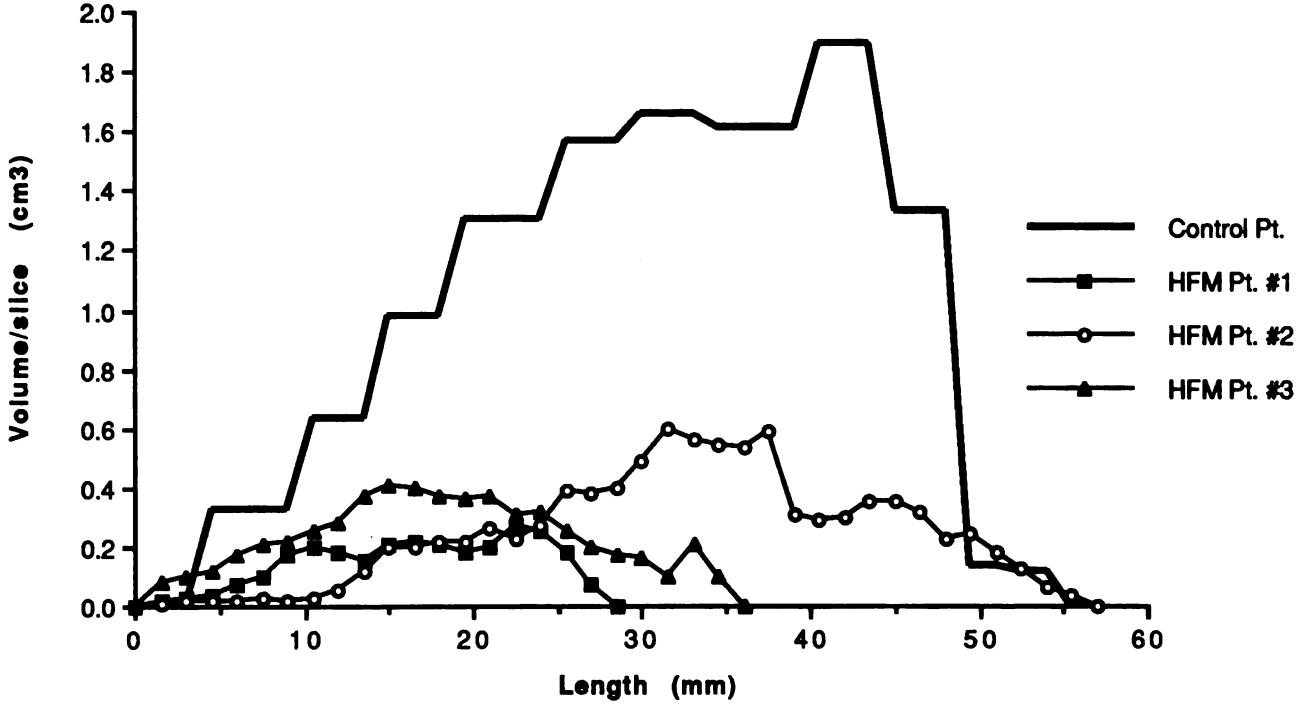


Figure 12d: Masseter, Post-Tx Contralateral Side, HFM vs Control

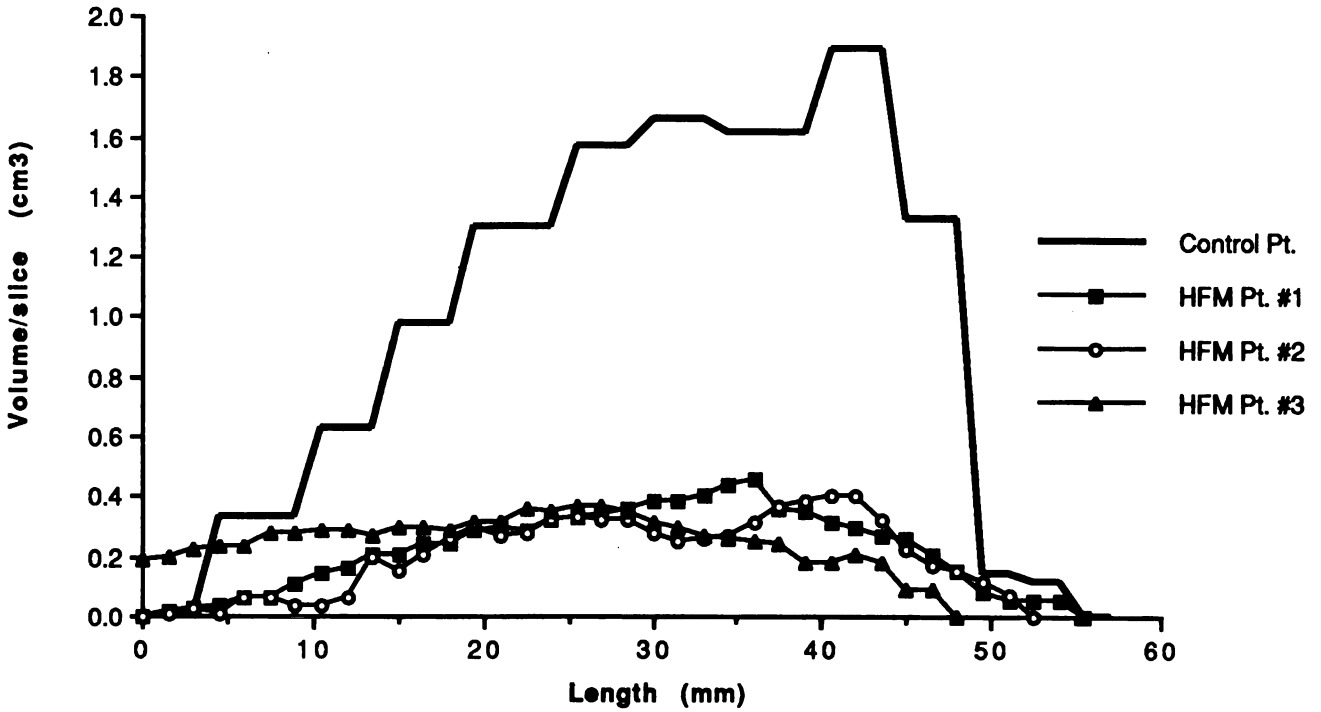


Figure 13a: Masseter, Changes in RCA as a Fxn of Growth and Tx, HFM vs Controls

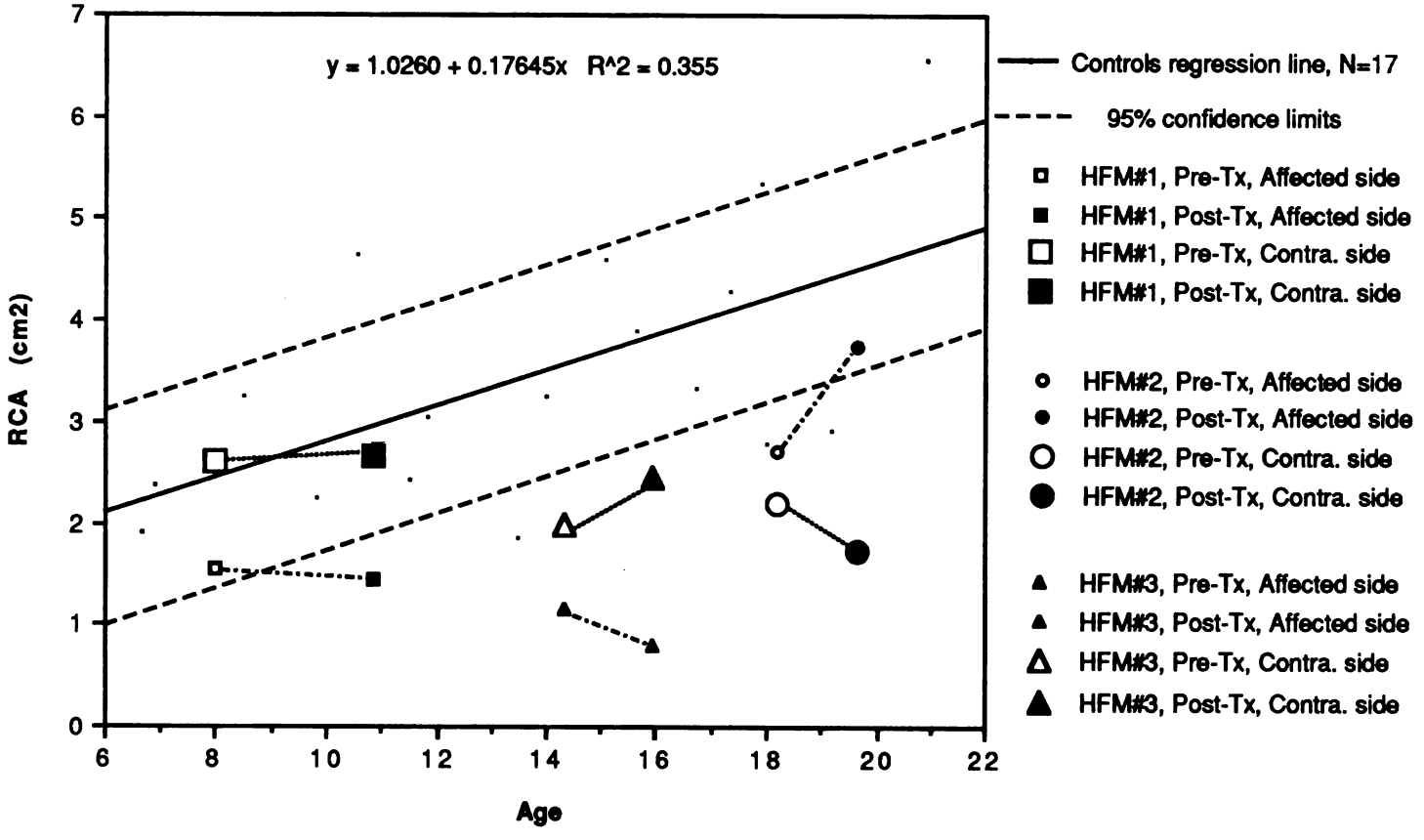


Figure 13b: Medial Pterygoid, Changes in RCA as a Fxn of Growth and Tx, HFM vs Controls

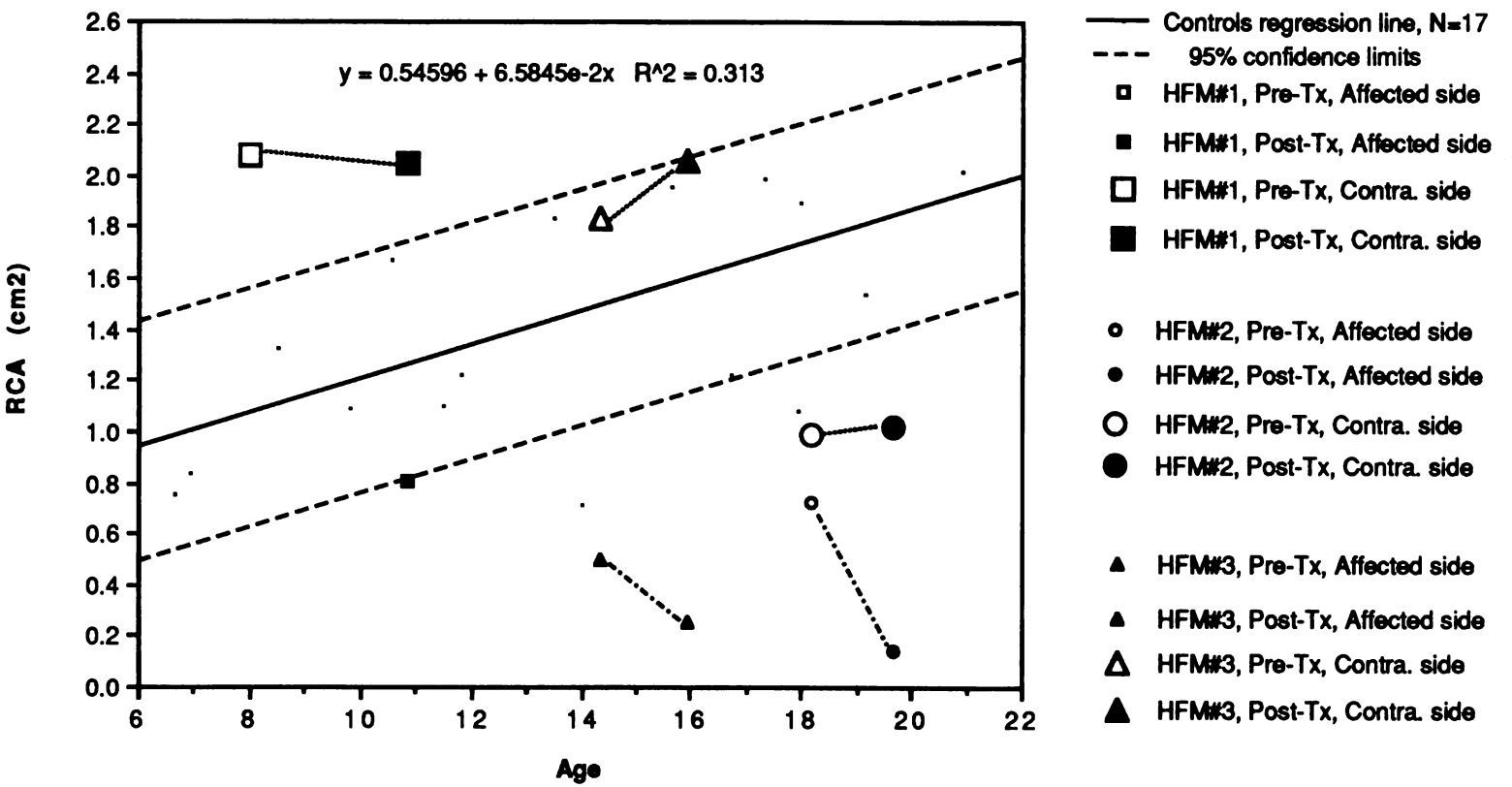


Figure 13c: Lateral Pterygoid, Changes in RCA as a Fxn of Growth and Tx, HFM vs Controls

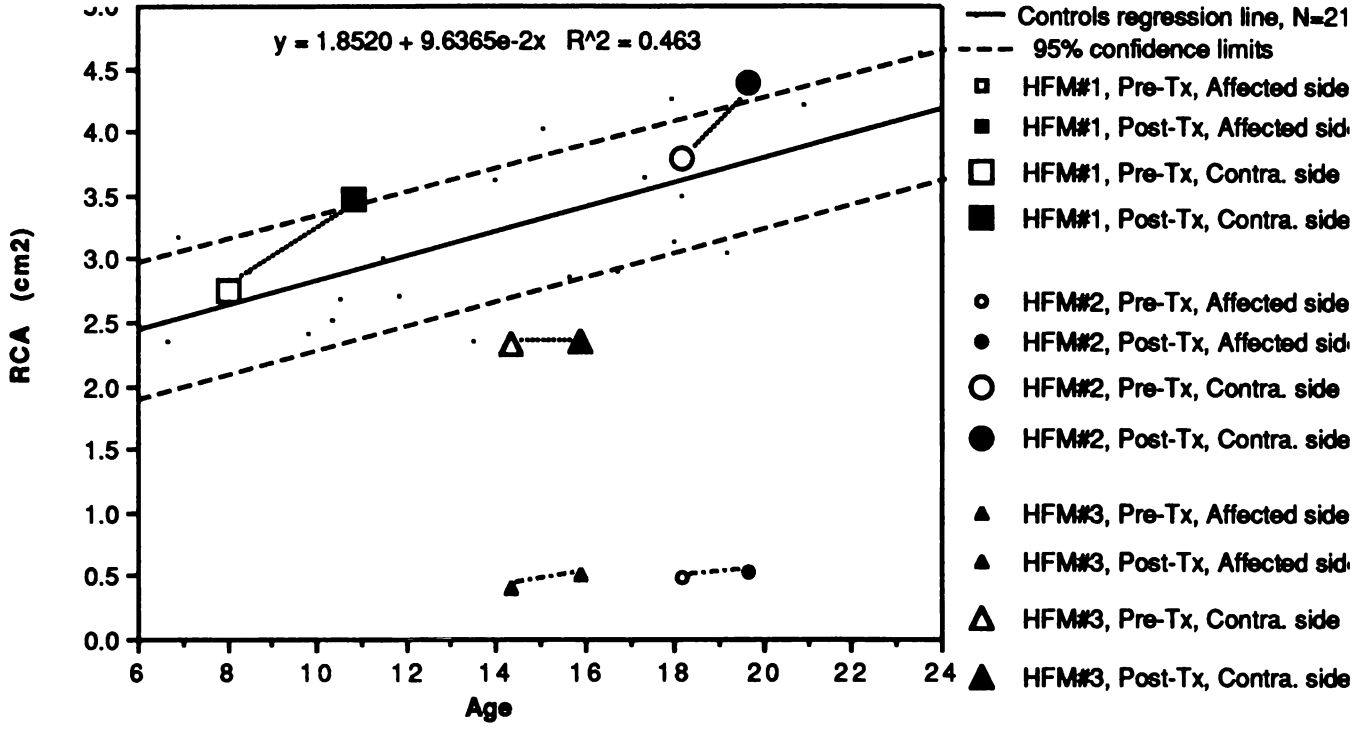


Figure 13d: Temporalis, Changes in RCA as a Fxn of Growth and Tx, HFM vs Controls

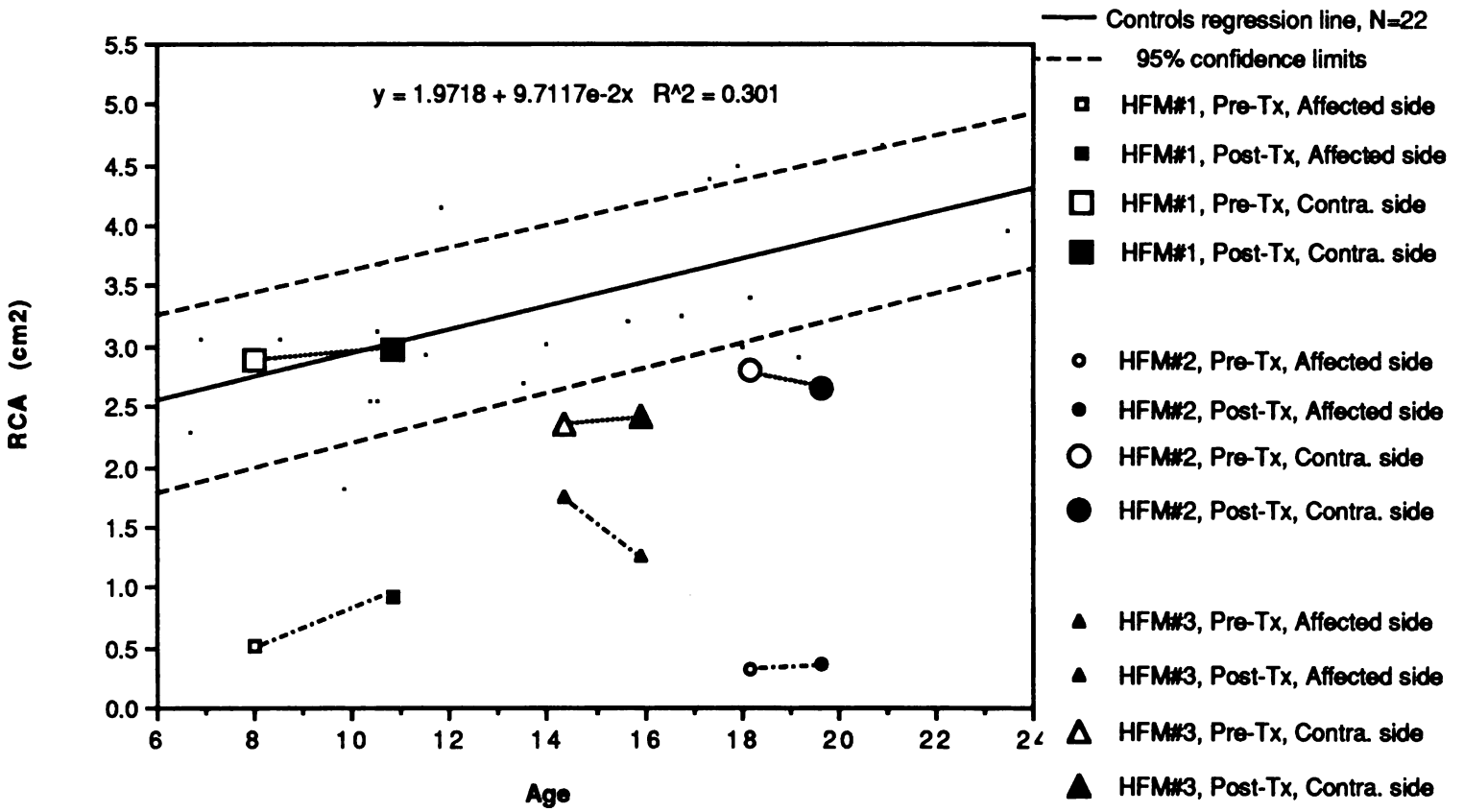


Figure 14a: Masseter, Changes in RepDen as a Fxn of Growth and Tx, HFM vs Controls

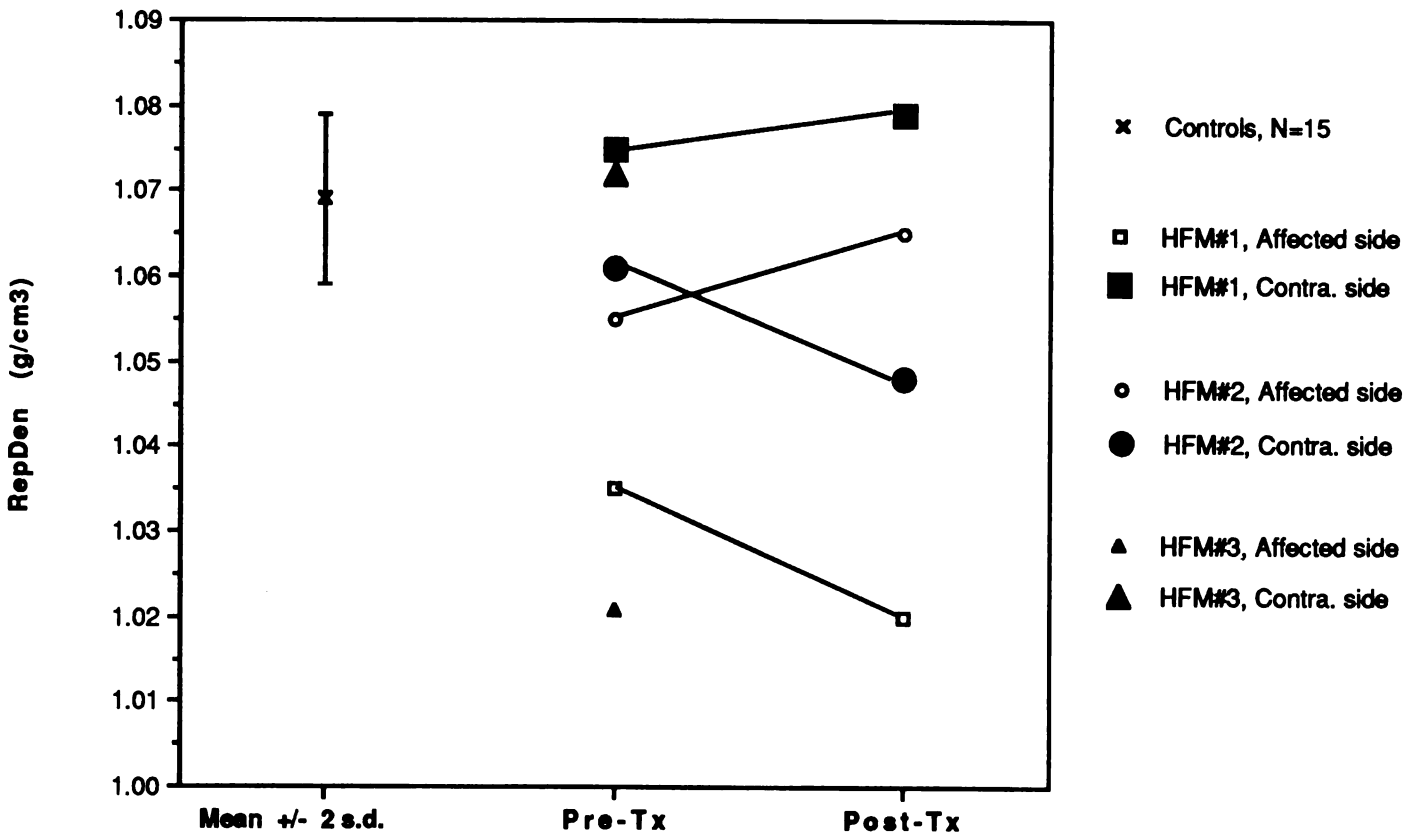


Figure 14b: Medial Pterygoid, Changes in RepDen as a Fxn of Growth and Tx, HFM vs Controls

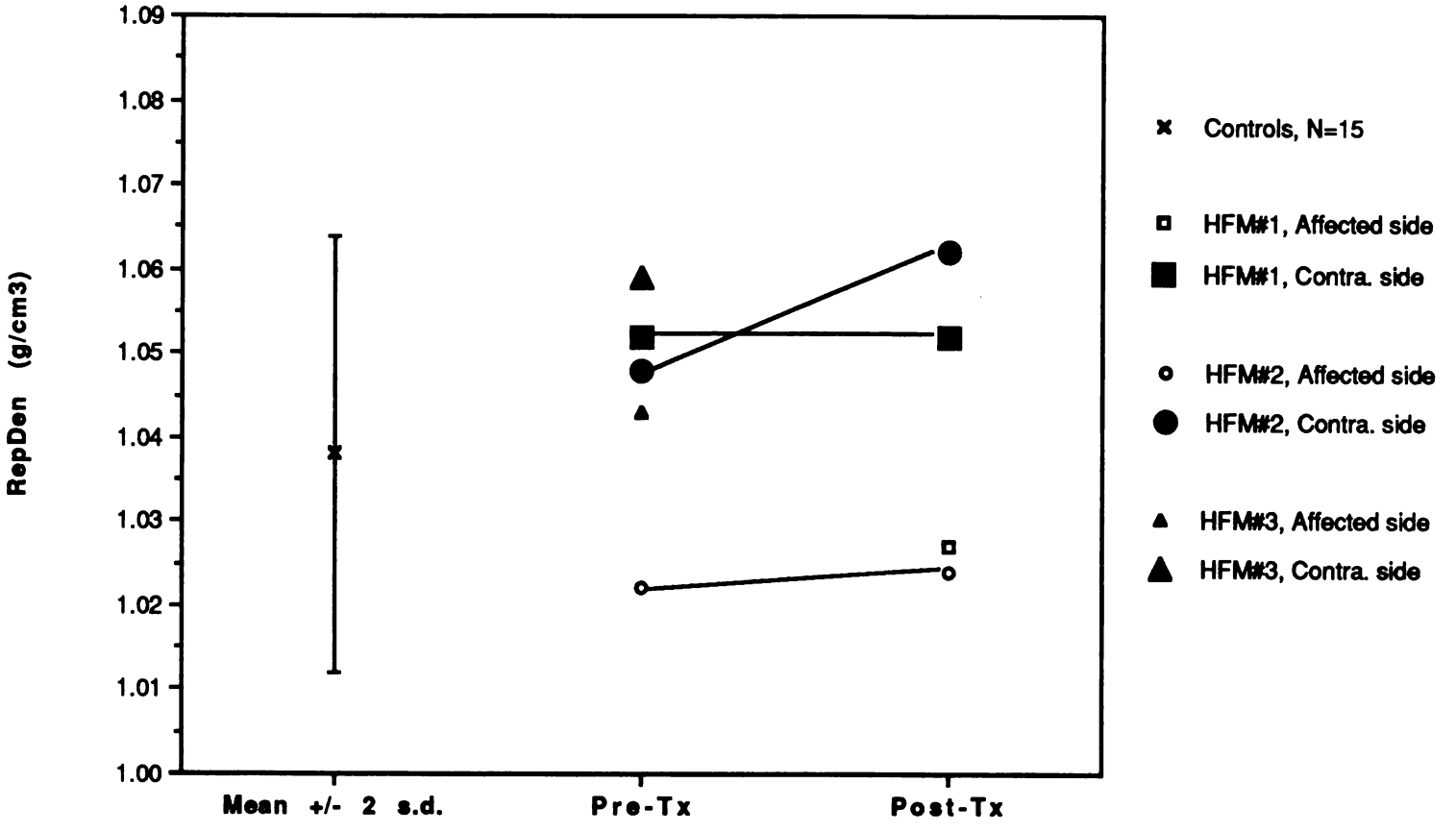


Figure 14c: Lateral Pterygoid, Changes in RepDen as a Fxn of Growth and Tx, HFM vs Controls

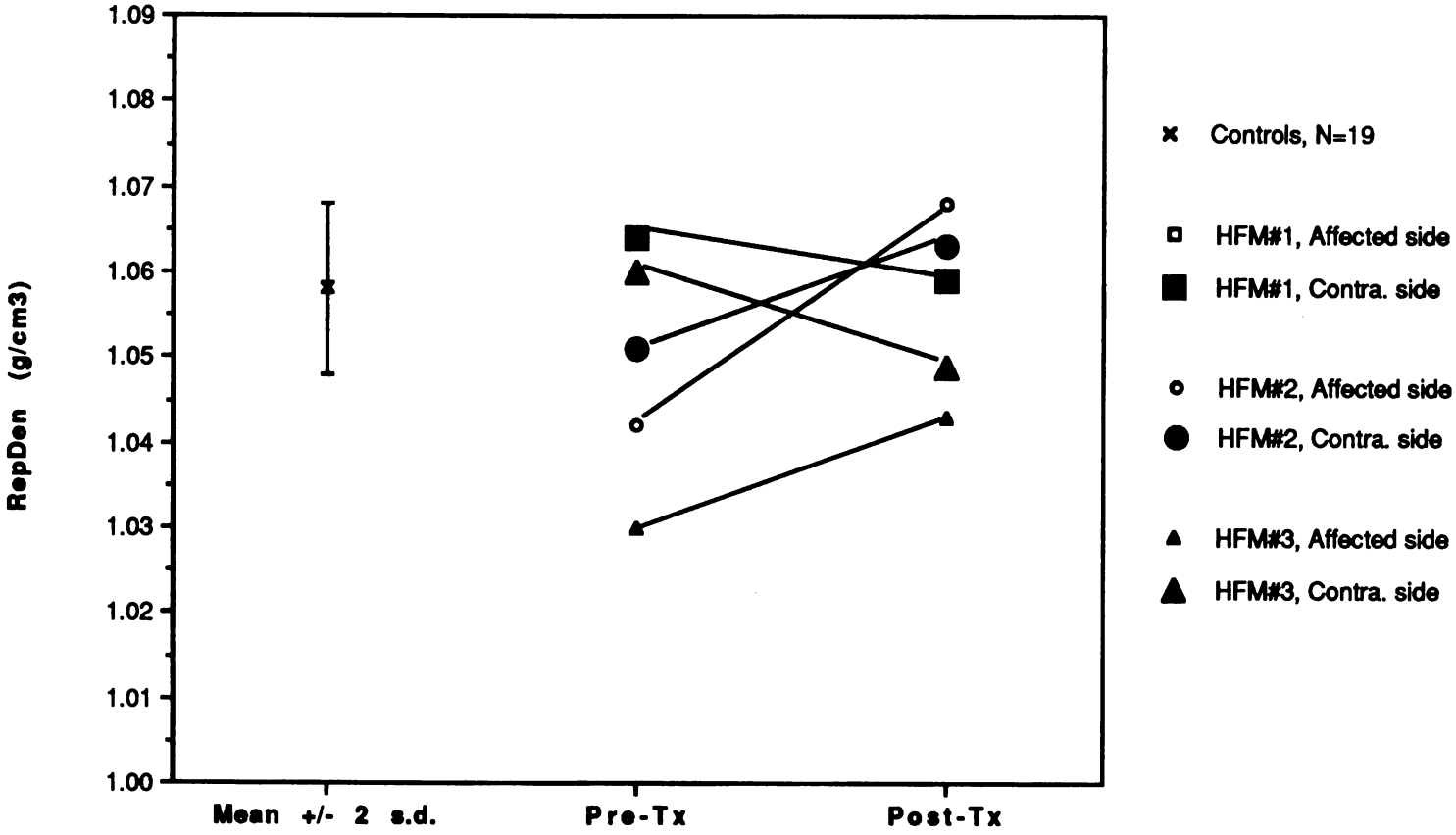


Figure 14d: Temporalis, Changes in RepDen as a Fxn of Growth and Tx, HFM vs Controls

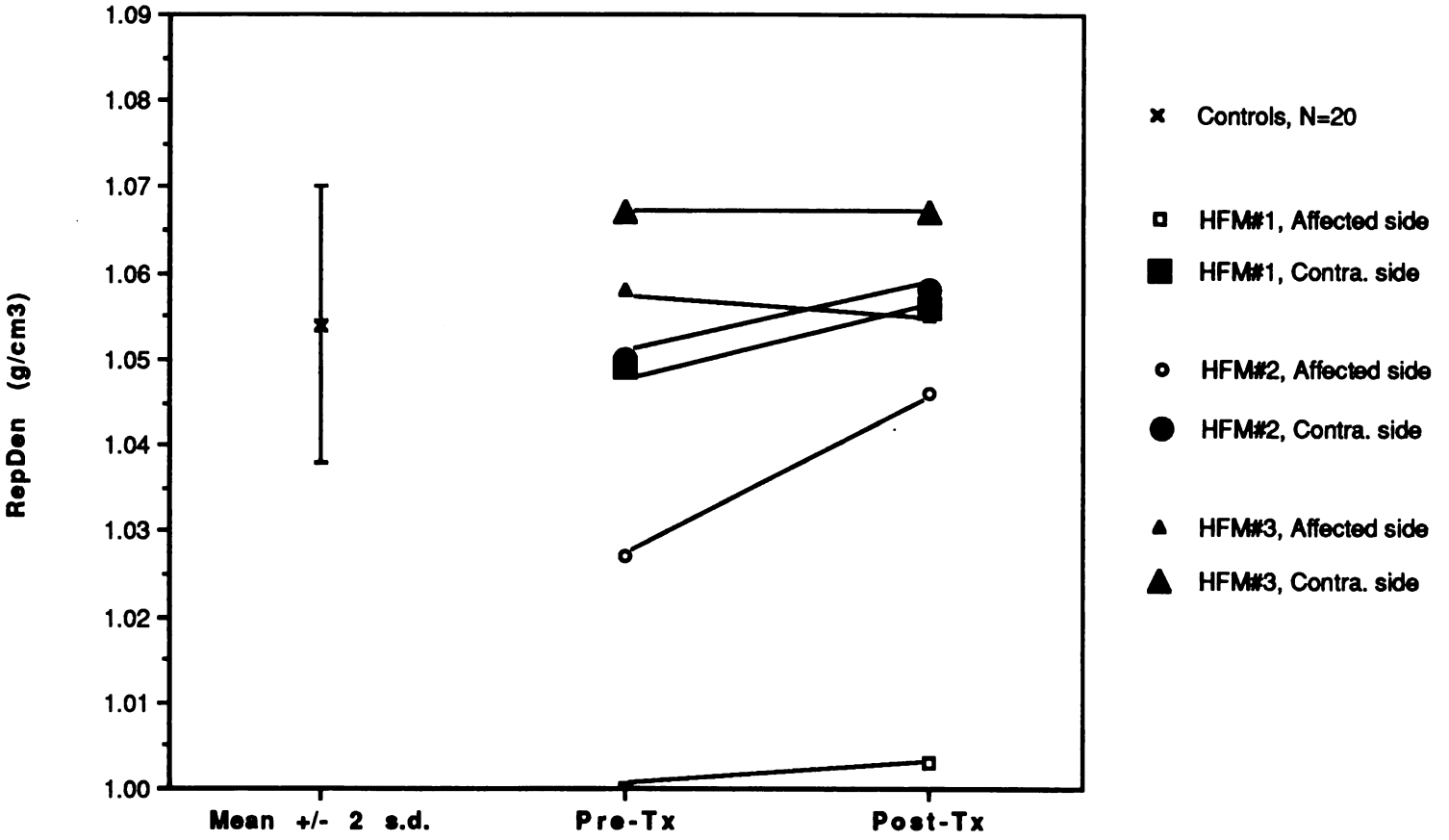


Figure 15: RCA Side to Side Variation, HFM vs Controls

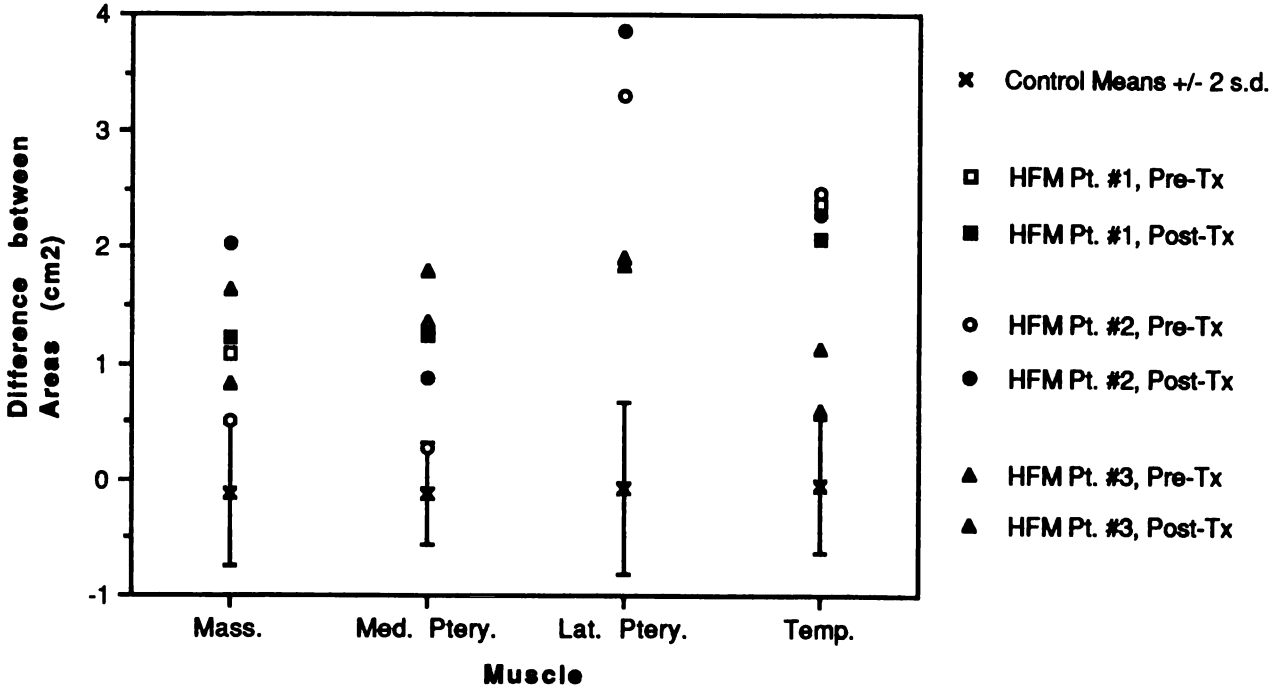
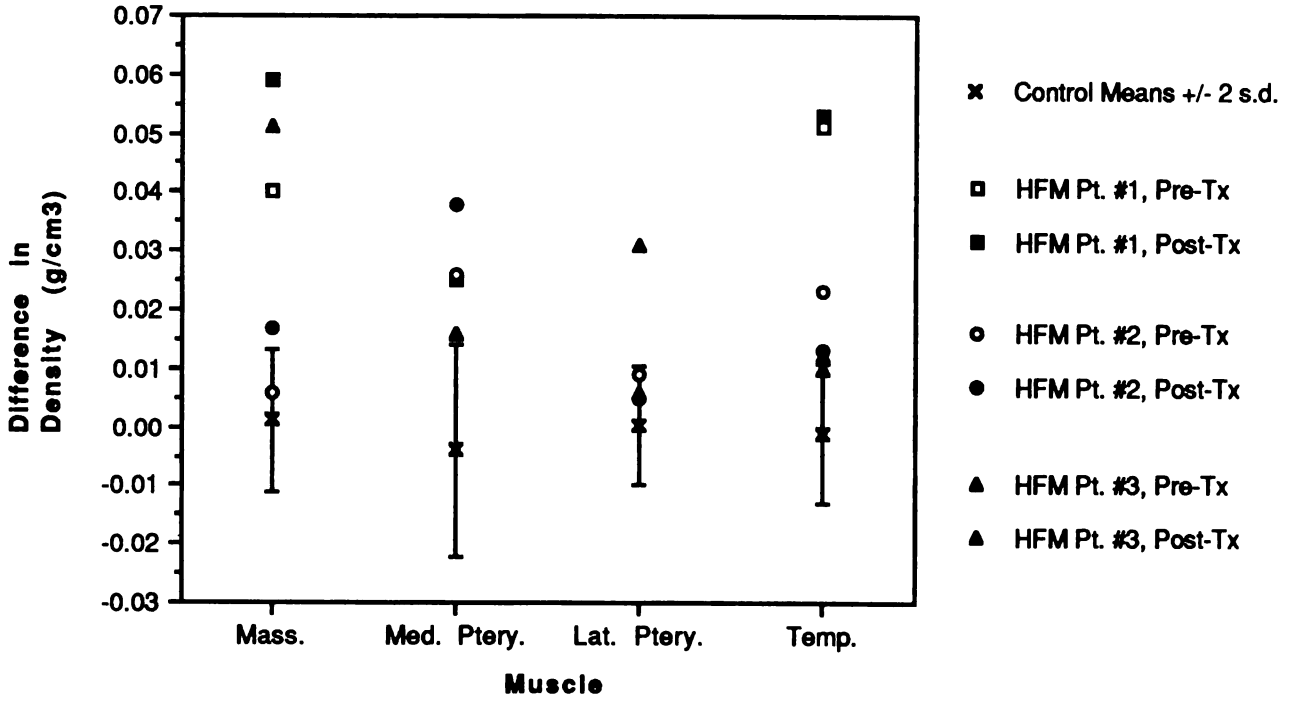


Figure 16: RepDen Side to Side Variation, HFM vs Controls



DISCUSSION

A. Part I: Standardization of Methods

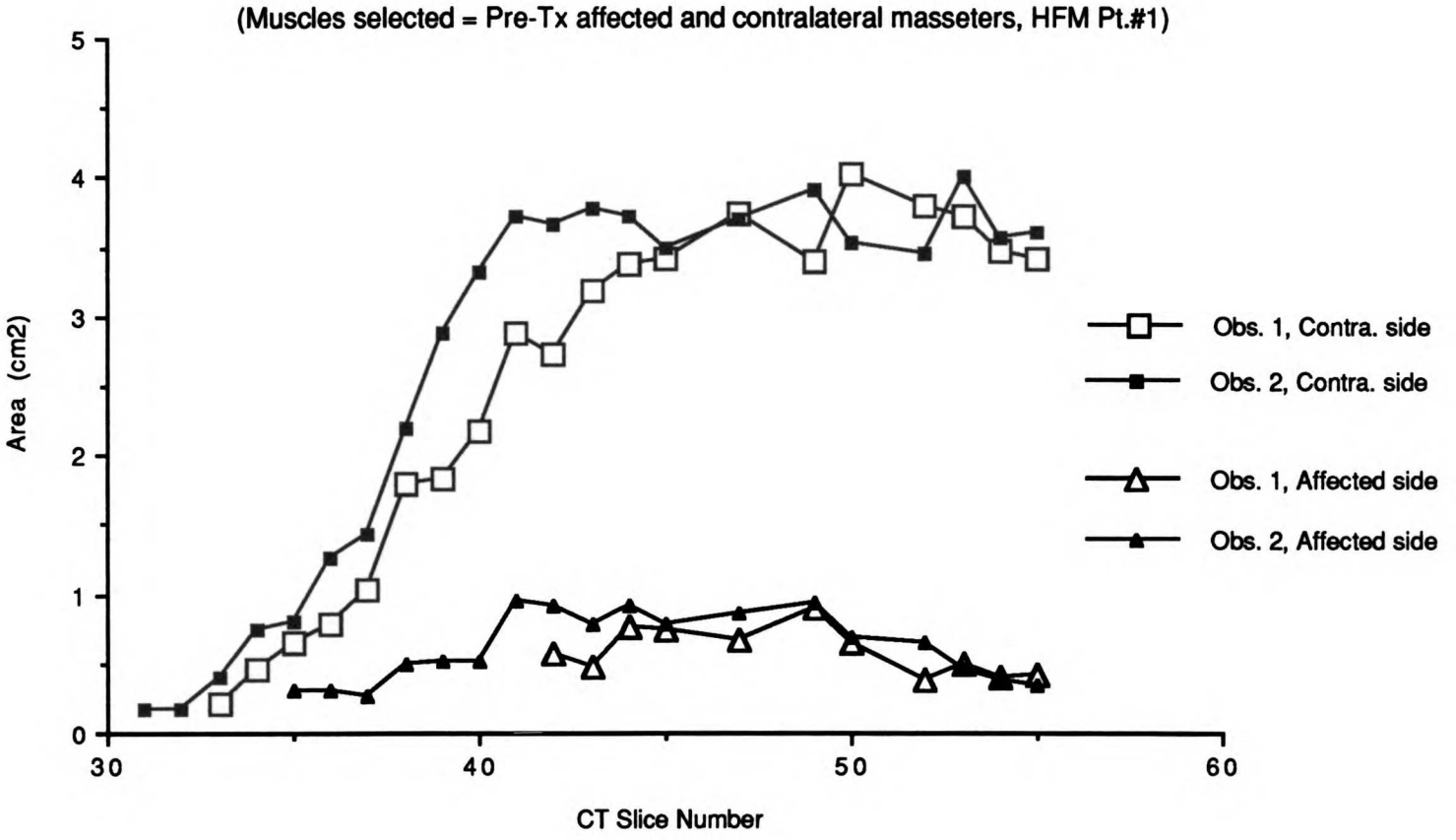
The first major challenge of this study was to develop and standardize the methods. More specifically, the measurement of quantitative changes in soft tissues occurring longitudinally and after therapy by QCT is still a fairly new technology. The standardization of the methods and the matched format procedure described in this thesis were important improvements over present available methods.

As referred to in the Methods and Materials Section, a number of alterations in the CT imaging protocol had to be reconciled during the course of this project. One of these was a variation in slice thickness, 3 mm thicknesses in some portions of the earlier CT scans versus a standard 1.5 mm later on. Another discrepancy was the variation in the superior and inferior limits scanned between pre- and post-treatment records. A third problem which arose was duplicate CT images in a single scanning session as a result of patient movement during the CT scan. The matched format procedure provided a method for reconciliation of these variations between each HFM subject's pre- and post-treatment scans. For example, if a muscle was not scanned in its entirety superiorly, then only images which could be matched between the pre- and post-treatment scans were used for QCT measurement. Likewise, duplicate images produced by patient movement were more easily identified during the matching process and could be removed from a data set. As for slice thickness variations, matching allowed observers, for instance, to pair one, 3 mm thick, pre-treatment slice to two, 1.5 mm thick, post-treatment images. Thus, equivalent regions-of-interest in pre- and post-treatment CT scans could now be and were compared.

The results of observer variability analyses demonstrate that QCT measurment variability was decreased through the standardization of QCT technology. One advantage of these tests was that both observers (1 and 2) had the opportunity to measure and remeasure identical records, under different conditions, standardized or not standardized. Theoretically, there should have been no differences between or within observers when measuring identical regions, regardless of the conditions. However, the results showed that standardization including the matched format procedure reduced the degree of measurment variability exhibited between and within examiners. This reduction was almost by a factor of 4x between observers and 6x within observers, as measured by the different standard deviations of the paired t-tests performed. Furthermore, the results showed the reduction in measurement variability was to a level which allowed the detection of small changes in muscle volumes between pre- and post-treatment time points. Thus, the hypothesis of Part I is accepted.

There was one other interesting finding concerning the observer variability tests that was discovered only when one examined the raw data. It was noted that observer 2 consistently measured each muscle area as slightly larger than observer 1, regardless of whether or not the conditions for both observers were standardized. An example of this is seen in Figure 17 which gives the area measurements slice by slice on the pre-treatment masseter muscles of HFM patient #1, as measured by observers 1 and 2. Note that observer 2 recorded areas as slightly larger than observer 1 did in virtually every instance. Hence, the data indicated that there was probably still some

Figure 17: Cross-sectional Area of 2 Muscles, as measured slice by slice by Observers 1 and 2



observer bias present even when conditions were standardized. A possible source of this bias is the tendency to trace images on CT monitors either on or just inside their borders. Thus, a limitation of this work is that standardization of the method alone did not result in complete agreement between observers.

B. Part II: Control Population

Another limitation of this work was the inability to obtain an ideal control group for comparison as defined in the Methods Section. It would have been convenient if the HFM contralateral side could have served as an adequate control as originally planned; however, Part IV showed that the contralateral muscles were different than those measured in a control sample.

Fortunately, an alternate control group, albeit with some limitations itself, was obtained. This control group did provide quantitative information about the size and density of masticatory muscles that has never been previously collected. The results clearly showed virtually no side to side variation in size or density exists in normal individuals, thus, proving the hypothesis of Part II.

Another objective of the control population was to provide some information about changes in muscle size and density as a result of normal growth and development. This was attempted by constructing regression analyses using control RCA and RepDen values, seen in the graphs of Part IV. The limitation of this approach was that it employed cross-sectional data which showed that growth occurred at a steady rate. This is probably not accurate for the individual case as most individuals have growth spurts at different ages. Growth curves should ideally be obtained from longitudinal data which unfortunately could not be obtained. However, this limitation had little effect with respect to muscle density, since it was discovered that there was very little variation in density as a function of age for the ages examined within this study (ages 6 to 23).

C. Part III: HFM Population

Some of the more interesting observations of this research were found in this part of the study. The pre-treatment volumes of the muscles of mastication of the affected side were found, on average, to be less than those volumes of the contralateral side. However, this finding was only statistically significant for the lateral and medial pterygoids and the temporalis muscles and not for the masseter muscles. These results quantified for the first time the asymmetric soft tissue growth associated with HFM. Furthermore, the affected masseters in two of the HFM subjects had the unusual qualitative finding of having an origin in an abnormal location, external to the affected zygomatic arch.

One other item concerning the affected masseter muscles should be pointed out. In all three HFM subjects the bone grafts were placed with the superior end being located in the area of the absent glenoid fossa. This resulted in the affected masseters lying on the anterior surface of the superior halves of these grafts. At no point did the grafts come near the inferior ends of the affected temporalis muscles, those muscles having terminated more superiorly in the infratemporal fossa. Thus, if any new masticatory muscle attachment occurred on the affected side post-surgically, it most likely involved the masseter muscle. In light of this information, it may be appropriate to utilize any HFM therapeutic measures which would encourage interactions between grafts and affected masseter muscles. Such interactions could result in a heavier bony structure as well as increase the possibility of hypertrophy of the affected masseters.

The second hypothesis of Part III stated that HFM therapy would increase both the size and density of affected HFM muscles as compared to contralateral side muscles. This hypothesis was rejected; however, a number

of interesting findings were seen in this section as well. During therapy the affected medial pterygoids did display a significant decrease in density as compared to their antimeres. They were also the smallest muscles in terms of length and volume, both pre- and post-treatment. It is likely that this decrease in density occurred because two of the three HFM patients (Type V sujects) never attained medial pterygoid insertions and thus, these muscles were probably always non-functional.

Significant findings were also seen in the lateral pterygoid muscles. The most notable finding was the absence of this muscle on the affected side of the two Type V HFM subjects. A possible explanation for this could be the intimate relationship between the lateral pterygoid muscle and the temporomandibular joint (TMJ). Perhaps since no lateral pterygoid muscles developed in these subjects, neither could the TMJ form. This finding also demonstrated that a significant difference existed between the Type V and Type IV patients, the latter possessing an affected side lateral pterygoid.

Of further note, the contralateral lateral pterygoids were the only contralateral muscles to increase in size during therapy. A possible explanation for this finding is that the lateral pterygoid is primarily responsible for protrusive movements of the mandible. Since other muscles do little to assist with this function and since the affected lateral pterygoids are missing or very diminuitive, the contralateral lateral pterygoids' response could be one of hypertrophy.

Some of the most exciting results of this study were the qualitative findings of Part III in the Results Section. The use of CT to examine HFM patients was a new approach. Previous works have relied upon clinical and 2-dimensional radiological assessments (Harvold, 1975, Chierici, 1983, Vargervik and Miller, 1983, Kaban, 1990). CT allowed us to make three-dimensional, in

vivo observations, which resulted in new discoveries. Improvements in our diagnostic skills utilizing CT will lead to better understanding of the nature of HFM and presumably to improved treatment modalities.

A couple of limitations also hampered this part of the study. The most obvious was the small number of subjects in the HFM population. As mentioned in Methods, large numbers of such subjects are hard to come by. Furthermore, three of the original six HFM patients selected for this study were disqualified. It is hoped that in the future subjects can be added to this study, not only at UCSF but at other centers as well, now that a protocol has been established.

Another limitation of this study was the small number of time points at which data could be collected. This is due to the fact that CT is not a totally benign procedure and efforts were made to keep the subject's radiation exposure to a minimum. One possibility to overcome this limitation in the future would be through the use of magnetic resonance imaging (MRI) instead of CT scans. In the past, MRI was not accessible and today it is still quite expensive. CT scans have the advantage of giving information on both hard and soft tissues, whereas, MRI is primarily useful with respect to soft tissue. On the other hand, MRI does not involve radiation and can thus be done more frequently. Furthermore, MRI has been shown to be as accurate as CT when measuring the cross-sectional area of jaw muscles with the exception of the lateral pterygoid muscle (Spronsen et al., 1989).

D. Part IV: HFM Subjects vs. Control Subjects

The objective of the first hypothesis of Part IV was to determine if the pre-treatment contralateral muscles of HFM were similar to control muscles. If the contralateral muscles were found to be normal, they could serve as controls for changes measured in HFM affected muscles. Toward this end, three separate analyses were used. In the first analysis a representative muscle, the masseter, was chosen (for reasons explained in Methods), to examine the categories of muscle length and volume. Muscle length was found to be quite similar between pre-treatment contralateral and control masseter muscles, however, volume was not. A criticism of this first analysis was that the control patient selected for comparison also had a diagnosis of chronic airway obstruction. Individuals with airway obstruction usually suffer from what some refer to as "long face syndrome," an increase in vertical face height development. This would tend to increase the size of this subject's muscles of mastication and possibly make him an inappropriate control. In addition, the age of this control subject was 7, whereas the ages of the pre-treatment HFM subjects ranged from 8 to 18 (Figure 4). Thus, if the control subject did have an increased vertical face height, it could explain why this 7 year old's masseter was already as long as an 18 year old HFM subject's masseter. Unfortunately, this was the only control patient in which it was possible to obtain a complete head scan.

The second and third analyses used the operationally defined terms of RCA and RepDen to further evaluate the similarity of contralateral muscles to controls in the categories of size (cross-sectional area) and density, respectively. A limitation of the second analysis was previously mentioned in Part II of this Discussion. This was in regards to the use of cross-sectional control data to establish what changes in RCA would occur with normal growth

and development. The second analysis displayed variable results with no clear trends. The small number of HFM subjects is partly to blame for this result. A greater sample size would be more likely to show a clearer tendency. The third analysis of density, on the other hand, demonstrated a significant degree of similarity between HFM contralateral and control muscles. Therefore, reviewing all the data, one is apt to conclude that the contralateral muscles of HFM are more dissimilar than similar to controls with respect to size but not with respect to density. Thus, they can not be considered completely normal.

The second and third hypotheses of Part IV were concerned with the amount of side to side variation seen in HFM subjects as compared to controls. With respect to muscle size, as measured by RCA, nearly all HFM masticatory muscles displayed a degree of pre-treatment side to side variation that was significantly greater than the controls. Most of the measurements of muscle density (RepDen values) also exhibited this result. The only significant exceptions that were seen to these results were in the masseter, medial pterygoid and lateral pterygoid muscles of HFM patient #2.

Hypothesis 3 stated that HFM therapy would normalize the amount of side to side variation in size and density seen in HFM muscles. With regard to size, virtually every case, instead, showed an increase in the amount of side to side variation following HFM treatment. Thus, the degree of asymmetry in size increased. The results concerning muscle density, on the other hand, were less clear. The masseter and medial pterygoid muscles again displayed an increase in side to side variation, whereas the temporalis muscles showed virtually no changes. However, the lateral pterygoid muscles in the one patient in whom they were present experienced a decrease in side to side density variation with post-treatment values falling in the range of normals. There were two possible explanations for this result. Either the affected

lateral pterygoids underwent an increase in muscle density or the contralateral lateral pterygoids experienced a decrease in muscle density during therapy. Data from Figure 14c showed that contralateral lateral pterygoids had RepDen values within normal limits before and after treatment. This graph also showed that the affected lateral pterygoids had pre-treatment densities that were significantly less than control norms, but that their post-treatment densities were within normal limits. Thus, it appeared that the side to side variation in lateral pterygoid density was normalized during the course of HFM therapy by means of an increase in the muscle densities of the affected lateral pterygoids. Therefore, hypothesis 2 can not be rejected on the basis of these data.

The majority of the data suggested that prior to HFM therapy there does exist a greater amount of variation between the two sides of HFM subjects versus controls, especially with respect to size. Hypothesis 3, on the other hand, was rejected; the only possible exception being in the category of lateral pterygoid side to side density variation which did improve over the course of therapy. Hence, these results provided some solid evidence that the degree of soft tissue asymmetry in HFM subjects is indeed greater than the side to side variation seen in a normal population and that, for the most part, this asymmetry increased during skeletal reconstruction and functional rehabilitation.

A limitation that applied to all of Part IV of this study, was that these results were based upon the two operationally defined values RCA and RepDen. Unfortunately, these representative measurements only looked at a small portion of the muscles being examined. This was necessary, as explained in the Methods, due to the constraints involved in obtaining a control sample.

The fourth and final hypothesis of Part IV stated that HFM therapy would increase the size and density of HFM affected muscles such that, post-treatment, these values would approach those of the controls. The same three analyses that were used in testing hypothesis 1 of Part IV were applied here as well. The first analysis, examining changes in affected masseters' length and volume over treatment, demonstrated a particularly interesting finding. Comparing Figures 12b to 12c, one got the impression that affected masseter muscles undergo shape change during therapy. Pre-treatment (Figure 12b) these masseters were short and appeared to have two or three peaks in cross-sectional area along their longitudinal axes. This was in direct contrast to the control masseter whose belly only contained one peak in cross-sectional area. An appropriate analogy for the shape of these affected masseters might be a folded up accordion. Post-treatment (Figure 12c), however, these affected masseters appeared to have lengthened and assumed a shape containing only one peak in cross-sectional area, more similar to the control. One can speculate from these results that the accordion-like affected masseters became attached to the bone grafts, which Part III pointed out they lie against, and were stretched out longitudinally. However, with respect to hypothesis 4, the length and volume of these affected masseters did not closely approach the control's values, with the exception of HFM patient #2's masseter length.

The second analysis was also one of size, that of cross-sectional area as measured by RCA. There was a high degree of inconsistency in the findings of this analysis. The large variation between HFM subjects with regards to the directions, amounts, and rates of change in their RCA values as compared to the controls made drawing any conclusions very difficult. The limitations of this work probably played an important role in creating this inconsistency. The small sample size of the HFM population was one such limitation. Possibly

more than one independent variable was another; for example, placing both HFM Type IV and V patients in the HFM sample. Evidence already presented in Part III suggests that these two HFM types may not be as similar as once believed. A third limitation would again have to be the use of the RCA values themselves which only looked at a small portion of the total size of the muscles being examined. Thus, considering the results, hypothesis 4 could not be accepted or rejected with respect to size as measured by RCA.

The third analysis examined changes in density, as measured by RepDen. As in the second analysis, the large amount of variation made drawing any conclusions difficult. There was one interesting finding, however, in this analysis concerning the control group. Regression analyses comparing control RepDen values vs. age revealed that muscle densities did not change with increasing age. In fact, for the control group, whose ages ranged from 6 to 23, muscle densities for each of the four muscles of mastication on average varied less than 1%. With respect to the fourth hypothesis though, in terms of density, the hypothesis could not be accepted nor rejected at this time.

In summary, all four parts of this study have yielded important new information. Part I has advanced the state of the art of QCT by introducing a standardized methodology for measuring soft tissue changes. Part II established a protocol for and began the collection of normative data on masticatory muscle size and density. Parts III and IV tested the new methodology and resulted in some interesting findings. An important lesson from Parts III and IV is that future studies are indicated. Increasing both the HFM and control populations size is needed and enlarging this study from a single center investigation into a multi-center project would most certainly enhance the results.

E. Future Directions

1. HFM Type I Patients

Another future study could look at HFM patients with a type I mandibular deformity. These patients have a mandible with a shortened ramus, atypical condylar shape, reduced temporomandibular joint space, and presumed absence of condylar cartilage and disc. Their treatment is very similar to the type IV and V patients with one important exception. Since they have a functioning condyle and coronoid process, all that is required is a lengthening of the mandible. Thus, their surgery consists of a stripping of the masseter and medial pterygoid muscle attachments followed by a horizontal sectioning of the mandibular ramus. Then, an interpositional bone graft to lengthen the ramus is placed and the wound is closed with no attempt to reattach the muscles. Thus, in these patients there is no positioning of new bone near previously unattached muscles. However, lengthening of the ramus would possibly cause a lengthening of the medial pterygoid and masseter muscles. Conversely, the position of the temporalis and lateral pterygoid muscles would be undisturbed. Thus, one could hypothesize changes in the former two muscles and not in the latter.

2. Potential for Animal Models

Future directions would also include the development of an animal model to test the hypotheses put forward in this thesis. The first hurdle would be to create an animal model with an HFM-like defect. Production of an otomandibular malformation in CS1 mice using triazene was discussed in the Introduction (Poswillo, 1973). More recently, Sulik, Cook, and Webster (1988) have shown that gestational-day-8 exposure of retinoic acid (RA) in mice also affects the first and second branchial arches, producing severe craniofacial

deformities quite similar to HFM. They found that timing the RA insult to when the epibranchial placodes were active resulted in mandibulofacial dysostosis-like syndromes. They proposed that the pattern of these malformations was related to the particular vulnerability of cells in the vicinity of normal programmed cell death. Their theory is that excessive cell death for which the embryo may not be able to compensate is a possible mechanism of craniofacial malformation. Unfortunately, Sulik's group also found that their exposed murine fetuses generally did not survive to birth due to cardiac malformations. Thus, although detailed histological and electron microscopic analyses during fetal development are possible, administering HFM treatment as described in this thesis to such mice and examining its effects is not feasible (Sulik et al., 1988).

In fact, in order to do any surgical manipulations on an HFM animal model, one most likely would need a primate model (Cynthia Cook, personal communication). Poswillo (1973) reported production of otomandibular deformities in *Macaca irus* monkeys by administration of 10 mg/kg of thalidomide by gastric intubation from day 25 to day 32 of development. This dose, he noted, produced similar defects in man when given over the same period of embryogenesis. Unfortunately, macaque monkeys are large animals in the adult state, expensive to house, and difficult to handle without sedation.

An alternative selection, however, is the common cotton-eared marmoset, *Callithrix jacchus*. An identical thalidomide regimen to that given to macaque monkeys was shown to also produce HFM-like otomandibular defects in marmosets (Poswillo, 1972). Furthermore, this study had 10 live births of deformed offspring from a total of 11 fetuses following thalidomide administration to 6 pregnant marmosets. In addition, marmosets are readily available, inexpensive to purchase and house, easy to manage on a human diet,

and weigh about the same as an adult rat. In fact, 8 adult marmosets may be caged in the space occupied by one adult macaque monkey (Poswillo, 1972).

The literature reveals one other lab studying the production of craniofacial malformations using teratogens on primates. Hendrickx and co-workers at the University of California at Davis have treated *Macaca* and *Papio* spp. with triamcinolone acetonide (TAC) between 21 and 43 days of gestation. The results were severe malformations of the central nervous system and cranium accompanied by craniofacial defects. They also noted a high incidence of prenatal deaths and stillbirths in the monkeys but no significant increase in the TAC treated baboons (Hendrickx et al., 1980).

Thus to carry out a detailed histological analysis of otomandibular defects on an animal model, retinoic acid treatment of mice would be a suitable approach. However, to perform surgical manipulations similar to those used in human HFM therapy on an animal model, one would need a larger and more viable species. The literature indicates that thalidomide treated marmosets may be an appropriate choice for such a study.

CONCLUSION

This research had a number of goals. One of its major accomplishments was the application of a fairly new technology, computed tomography, to studying changes in soft tissues after surgical intervention and functional rehabilitation. Toward this end, a new standardized methodology was introduced and shown to decrease observer measurement variability. This new methodology could be used by other centers doing similar research. A second achievement was the first collection to date of normative data on masticatory muscle size and density. This information can be useful not only for comparison to HFM, but also for any other craniofacial anomalies such as Treacher Collins syndrome. Thirdly, this work showed quantitatively for the first time the amount of soft tissue asymmetry that exists in HFM. In addition, numerous, previously unreported, qualitative findings observed in the HFM subjects were also documented.

Furthermore, this research demonstrated that the contralateral side of HFM can not be considered completely normal and unaffected, especially with regard to size. Finally, this study did not show that the hypotrophic muscles of HFM undergo normalization in response to altered skeletal morphology and functional rehabilitation as compared to controls. Further work needs to be done in this area; more HFM patients need to be studied and other centers of HFM treatment need to be involved. In addition, the development of an animal model, as detailed in the Discussion Section, could be helpful.

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APPENDIX A

Forms

HFM STUDY: MASTER PAGE FOR INITIAL DATA									
Patient_____DOB_____ Surg. type/date_____ Observer_____									
Legend: 1=masseter, 2=medial pterygoid, 3=lateral pterygoid, 4=temporalis, R=right, L=left									
DFOV=	Pretreatment CT Scan Date_____				Posttreatment CT Scan Date_____				DFOV=
CombRun#=	Tape #'s/Run #'s_____				Tape #'s/Run #'s_____				
Combi-Slice#	Old slice#	level, mm	Muscles	Comments	Old slice#	level, mm	Muscles	Comments	

HFM STUDY: INITIAL DATA COLLECTION

Patient _____ DOB _____ Surg. type/date _____ Observer _____

Pretreatment CT Scan Date _____

Tape #'s/Run #'s _____

Window _____ Level _____

Posttreatment CT Scan Date _____

Tape #'s/Run #'s _____

Window _____ Level _____

Slice	Level	R.		L.		Comments	Slice	Level	R.		L.		Comments
		Area,cm^2	CT# (HU)	Area,cm^2	CT# (HU)				Area,cm^2	CT# (HU)	Area,cm^2	CT# (HU)	


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