Magnetic Resonance Spectroscopy of the Human Masseter Muscle in Nonbruxing and Bruxing Subjects

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The masseter muscles of six nonbruxing subjects (five men, one woman) and six bruxing subjects (four men, two women) were assessed during chewing by nuclear magnetic resonance spectroscopy (^{31}P-NMR). The NMR spectra were collected on a GE Sigma 1.5T whole body magnet with a double-tuned ^{31}P/^{1}H surface coil. Two-minute trials of rest/chewing/rest were completed three times. Averaged spectra of inorganic phosphate, phosphocreatine, and three adenosine 5' triphosphate peaks were collected in each trial. Bruxing subjects had a lower concentration of total phosphate and phosphocreatine than nonbruxing (control) subjects at rest. Bruxing subjects increased their inorganic phosphate during chewing significantly less than control subjects. The pH levels during rest and during chewing were similar in both controls and bruxers. These preliminary results suggest that bruxing subjects exhibit an altered phosphate metabolism during rest and exhibit a different phosphate metabolism pattern during chewing as compared to nonbruxing subjects.
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Bruxing involves an increased use of the craniomandibular muscles in clenching and grinding patterns. Bruxing can be associated with pain in these muscles but is not a compulsory finding.[1–6] Despite advances in studies of muscle pain, much is still not known about chemical changes in muscles associated with pain.[7–9] Nuclear magnetic resonance spectroscopy (in vivo ^{31}P-NMR) provides a noninvasive tool to assess muscle metabolism. It seems appropriate to apply magnetic resonance spectroscopy to study muscle metabolism in subjects who do not brux, subjects who brux, and subjects who brux and have pain in these muscles.

The masseter was chosen because it constitutes a jaw-closing muscle of the mandible involved in parafunctional habits. The masseter was examined by ^{31}P magnetic resonance spectroscopy at rest, during three cycles of chewing, one cycle of intercuspal clenching, and recovery. Based on studies of athletes' limb muscles,[10,11] it was hypothesized that the subjects with the parafunctional habit of bruxing would enhance the activity of the masseter at a sufficient level to alter the metabolism of the muscle fibers. There have been few studies of the metabolites in the craniomandibular muscles, and most prior studies of the metabolism of skeletal muscles have used repeated biopsies and chemical analysis.[12–15] Many of the metabolites involved in cellular chemical energy transfer in muscle contain phosphorus and occur freely in solution at concentrations high enough (0.5 mmol/kg) to be detected by ^{31}P-NMR.[16,17] While

the biopsy studies analyze the changes in several metabolites, including phosphate, ^{31}P magnetic resonance spectroscopy allows a noninvasive technique with repeated in vivo measurements during prolonged exercise.[18-20]

The ^{31}P magnetic resonance spectroscopy is used to estimate:

1. Adenosine 5' triphosphate (ATP), the primary energy chemical of the muscle cell
2. Phosphocreatine (PCr), a readily available chemical source to replace ATP
3. The physiologic concentrations of inorganic phosphate (Pi) indicative of the chemical breakdown of ATP during the development of myosin cross-bridges

Pilot work by Cohen et al[21] on subjects with muscle pain suggests that the Pi/PCr ratio in these patients alters during chewing. This finding suggests that subjects with muscle pain do exhibit unusual metabolic properties of at least one craniomandibular muscle.

The ^{31}P magnetic resonance spectroscopy measures the relative concentrations of the unbound forms of these molecules as well as the change in pH in the muscle fibers when the Pi peak shifts. The human masseter muscle provides a bimodal distribution of two primary groups of fibers: about 70% of the fibers are the oxidative type I; 25% are the glycolytic type IIB. The masseter should provide an excellent study of correlating the pH changes with the type of fibers.[22-25] Recent studies on the human masseter during clenching indicate that the pH changes depend on the region of the masseter studied.[26] Also, the Pi/PCr ratio significantly increases when an individual clenches on a first molar.[26,27] Lam and Hannam[26] have shown that the Pi/PCr ratio in normal subjects clenching on a bite transducer will increase more than 2.5 times the resting level and that the pH will decrease from about 7.14 to 7.01, depending on the region of the masseter.

Some authors studying skeletal muscles have attempted to find whether a correlation exists between phosphate metabolism and pain in a muscle. In experimental studies inducing pain, subjects performed repeated exercises involving lengthening while contracting and then were followed over 2 days.[28] The baseline Pi/PCr ratio (0.12 ± 0.01) appeared to elevate within 1 hour following the repeated lengthening contractions of the human arm or leg muscles. The maximum increase had occurred by 1 day after the exercise (0.21 ± 0.05). Eccentric high-force contractions of the quadriceps muscles of the human leg have shown that starting within 2 days of the exercise, the myosin from the slow contracting muscle fibers increases its concentration in the muscle circulation. This is correlated with changes in the magnetic resonance image intensity.[29] Further work relating muscle pain in the craniomandibular muscles applying magnetic resonance imaging and spectroscopy is needed.

Materials and Methods

Subjects

Six nonbruxing (control) subjects and six bruxing subjects were selected. The control subjects consisted of five men and one woman with a mean age of 28.0 ± 0.8 years. The six bruxing subjects in the experimental group consisted of four men and two women with a mean age of 28.0 ± 1.0 years (Table 1). Of the experimental group of six patients, three of the bruxing patients exhibited no pain in the masseter muscle while the other three patients had pain within the masseter muscle at the time of the ^{31}P-NMR study.

Screening Tests

Subjects were selected at an initial clinical screening in which the study was described and a written consent was received after oral and written presentation of the complete study. The study had received prior approval from the Human Research Committee. Subjects then answered questions about their parafunctional habit and medical history. Presence of pain in the head and neck region was assessed by three methods. The patients were asked to indicate whether pain was present in selected regions and rate it on a linear scale of 1 to 10, with 10 as the most severe pain (Table 2). The patients also completed a profile chart indicating the sites where pain was present in the head and neck. The clinician examined the patient to assess mandibular movements and to determine if pain was present in the craniomandibular muscles by palpation with an algometer (Model PTH-AF2, Pain Diagnostics and Thermography, Great Neck, NY). Dental impressions were taken to verify presence of wear facets as a further method to accurately assess this sample group. Magnetic resonance images of both temporomandibular joints (TMJs) were used to assess the condition of the joints both in terms of the condyle and the disc. Only one subject, a control subject, demonstrated any type of internal derangement with disc displacement on closing.

Table 1 Control and Bruxing Subjects

	Sex	Age	Occlusal wear* (0–3)	Joint condition	Mandibular movements	Joint noises	Bruxing level† (0–2)
Control subjects							
1. (WA)	M	28	0	—	Normal	None	0
2. (CC)	M	29	1	—	Normal	None	0
3. (HF)	M	28	1	Disc displaced on closing	Normal	None	0
4. (WH)	M	27	1	—	Normal	None	0
5. (GN)	M	27	1	—	Normal	Rt: early opening click	0
6. (CO)	F	27	1	—	Normal	None	0
Bruxing subjects							
7. (SA)	M	29	1	—	Opening to left	Rt: early click	2
8. (TM)	M	28	1	—	Normal	None	1
9. (SS)	M	27	1	—	Restricted opening	Pop on wide opening	2
10. (KB)	M	28	1	—	Normal	None	2
11. (LM)	F	28	1	—	Normal	None	2
12. (WW)	F	26	1	—	Opening deviates	Lt: click on opening Rt: click on closing	2

*Occlusal wear rated on scale of 0 to 3: 0 = no wear; 1 = mild; 2 = moderate; 3 = severe.
†Bruxing level rated on scale of 0 to 2: 0 = no bruxing; 1 = occasionally bruxes; 2 = often bruxes.

Table 2 Site of Pain by Patient Report, Patient Profile, and Clinician Palpation

						Muscle pain			
						Profile*		Palpation†	
	Headache pain	Face pain	Neck pain	Tooth pain	Jaw joint	Temp	Mass	Temp	Mass
Control subjects									
1 (WA)	0	0	0	0	0	0	0	0	0
2 (CC)	0	0	0	0	0	0	0	0	0
3 (HF)	0	0	0	0	0	0	0	0	0
4 (WH)	0	0	0	0	0	0	0	0	0
5 (GN)	0	0	0	0	0	0	0	0	0
6 (CO)	0	0	0	0	0	0	0	0	0
Bruxing subjects									
7 (SA)	0	0	0	0	0	0	0	0	0
8 (TM)	0	0	0	0	0	0	0	0	0
9 (SS)	0	0	0	0	0	0	0	0	0
10 (KB)	0	3	2	0	0	0	+	0	+
11 (LM)	4	3	4	1	6	0	+	0	+
12 (WW)	2	1	3	0	2	0	+	+	+

First five questions based on patient designating severity of pain on a linear scale from 0 to 10, with 0 as no pain and 10 as most severe.
* Regions designated by the patient as having pain.
†Regions that the clinician determined had pain by applying punctate pressure with an algometer (0 = no pain; + = pain present).

Instrumentation

Magnetic resonance images and spectroscopy were completed with a GE Sigma 1.5 Tesla whole body unit (Fremont, CA) located at the Magnetic Resonance Research Center, University of California, San Francisco. Prior to the study, subjects were provided with a written consent form that explained the magnetic resonance recording session and specific safety factors. Subjects were placed in a supine position to carry out imaging of the TMJs. Two head surface coils, 7.6 cm in diameter, were placed bilaterally over the TMJs,

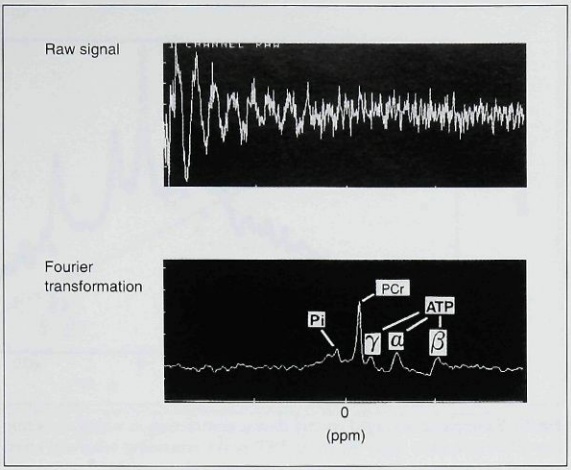

Fig 1 Example of the original ^{31}P-NMR signal *(top trace)* and the Fourier transformation of that signal into averaged spectra representing unbound phosphate in various chemical forms in a resting human masseter muscle. Five spectra appear in the normal signal with the first representing inorganic phosphate (Pi), the second phosphocreatine (PCr), and the last three the different phosphates of ATP. In the resting masseter muscle, the PCr peak is the largest and will decrease during contraction of the muscle. The PCr spectra are set at 0 for reference.

and the subject was requested to lie quietly maintaining his/her mandible in a resting posture. The TMJs were imaged twice with the subject sitting quietly in mandibular resting posture, then with the condyle forward as the subject held a 20-cc syringe between his/her teeth. At the completion of the imaging of the TMJs for later assessment of their condition, the two head coils were replaced with a smaller oval-shaped coil, 2 × 3 cm in diameter, placed over the belly of one masseter muscle. The left masseter was chosen for study in most subjects, except for three bruxing subjects who exhibited pain in at least one masseter muscle; the muscle with most pain was chosen for those subjects.

Exercise Protocol

Subjects were asked to place one piece of chewing gum (Trident) in the mouth on the side of the masseter to be tested, then to lie quietly for two initial 2-minute baseline recordings. Then they chewed unilaterally on the side of the tested muscle for 2 minutes, then rested quietly while keeping the gum in the mouth for 2 minutes. This was repeated for three trials. Next the subject clenched on his/her teeth in their intercuspal position at a maximum level for a 30-second period, followed by a final 2-minute recovery period. The protocol involved 10 trials consisting of six rest trials, three chewing trials, and one clenching trial.

Magnetic Resonance Spectroscopy Measurements

At the onset of the exercise trials, the magnetic field was adjusted for maximum proton resolution (0.4 ppm), and the magnetic field homogeneity was adjusted by shimming on water. The proton spectrum obtained at 80.3 MHz was used to calculate the percent lipid content from the total water and lipid protons in the masseter. The surface coil was shimmed using the proton frequency until *(1)* baseline resolution occurred for the ATP-gamma resonance, *(2)* a line width of less than 1 ppm occurred for the resting PCr, and *(3)* a signal-to-noise ratio of 30:1 was obtained for the PCr resonance. The PCr width was 42.5 Hz, and the sweep width was 2,000 Hz. Spectra were corrected for saturation effects (ie, reduced signal) at the start of the protocol before the first baseline recording with the muscle at rest.[30,31] The NMR spectra were obtained using a one-pulse sequence of P_1-A-D_1 (P_1 = the radio frequency excitation pulse length of 45 microseconds, A = the acquisition time lasting 0.512 seconds, and D_1 = the delay time lasting 2 seconds) (Fig 1). The spectra were obtained every 2 seconds with averages displayed after 16 repetitions in 32 seconds. The final averaged data, displayed at 2 minutes, consisted of 64 total spectra for 9 of the 10 trials, but the data from clenching consisted of a 32-second average of 16 individual spectra. The resonance heights and areas from the averaged data for each trial were determined and

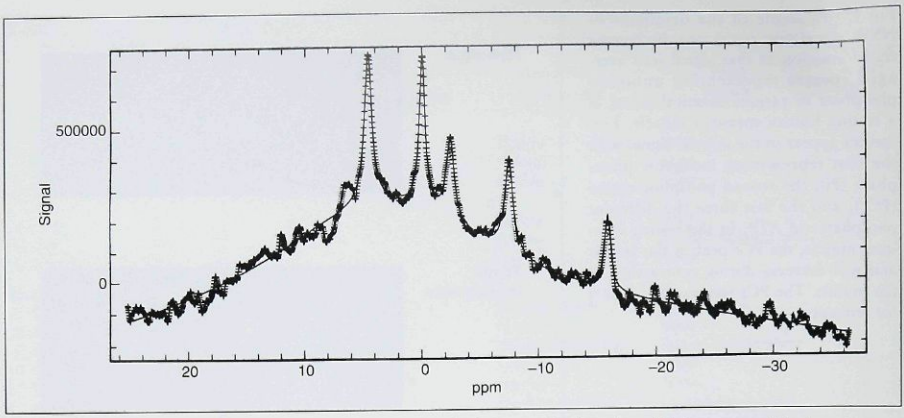

Fig 2 Example of averaged spectra during contraction in which inorganic phosphate (Pi) increases while phosphocreatine (PCr) decreases with the three ATP peaks remaining relatively constant. A Laurentzian curve-fitting program is applied to the spectrum to determine area and peak.

compared using a nonlinear least-square curve fitting program (Laurentzian curve fitting; Fig 2). Data were expressed as ratios to the ATP-beta peak of ATP, which was relatively stable based on previous studies of other muscles by other investigators[32,33] and on analysis by the authors of the present study. The areas of the various resonances are proportional to the quantities of the various metabolites in the volume of the surface coil, provided saturation does not occur. Previous studies by other investigators have shown that the area of the ATP-beta peak of ATP in control subjects at rest is equivalent to 5.5 mmol of ATP per kg of wet weight of muscle.[34] Due to the difficulty of identifying the baseline and rest Pi spectra for area measurements in some subjects, the peak values of each of the Pi, PCr, and ATP-beta spectra were used for analysis. Comparison of the two measures of the spectra indicated a strong linear Pearson's correlation coefficient (r = .83) between the area and peak measurements (Fig 3).

The chemical shift of Pi with PCr set at zero was used to estimate pH, as based on previous studies.[35–38] The Pi spectra are very sensitive to hydrogen ion concentrations. Thus, previous work on the frog muscle at 4°C, and in the rat brain, have provided pH values and constants for diphosphate ($H_2P0_4^-$) and monophosphate ($HP0_4^{2-}$). Since most of the inorganic phosphate (Pi) remains in the muscle fibers, the shift in spectra between Pi and PCr provides a fairly accurate measure of intracellular changes in hydrogen ion concentrations that can be projected into a logarithmic relationship:

$$pH = 6.72 + \frac{\log_{10}(d - d^1)}{(d_2 - d)}$$

where d is the chemical shift (in ppm) of Pi as related to PCr (ie, 5.0 ppm – 0.2 ppm), d_1 is the chemical shift of $H_2P0_4^-$ set at 3.27, and d_2 is the chemical shift of $HP0_4^{2-}$ set at 5.69. Based on these numbers, a chemical shift of Pi of 5.0 ppm would equal a pH of 7.1, and a 4.3 ppm shift would equal a pH of 6.59. The pH values were calculated for each of the 2-minute test periods, and the mean and standard deviations were determined for data in each of the two groups.

Statistical Analyses

Absolute values of the phosphate chemicals would depend on the absolute volume that contributes to the signal minus spurious inputs from tissue such as bone. Instead, the present study used relative changes in the concentration of phosphorus metabolites based on both the heights and areas under the ATP-beta peak as the reference. Then the heights or areas of Pi, PCr, and all five peaks to the ATP-beta spectra were compared, and the standard Pi/PCr ratio was used. Data compared between the control group and the experimental group (ie, total phosphate) were subjected to a

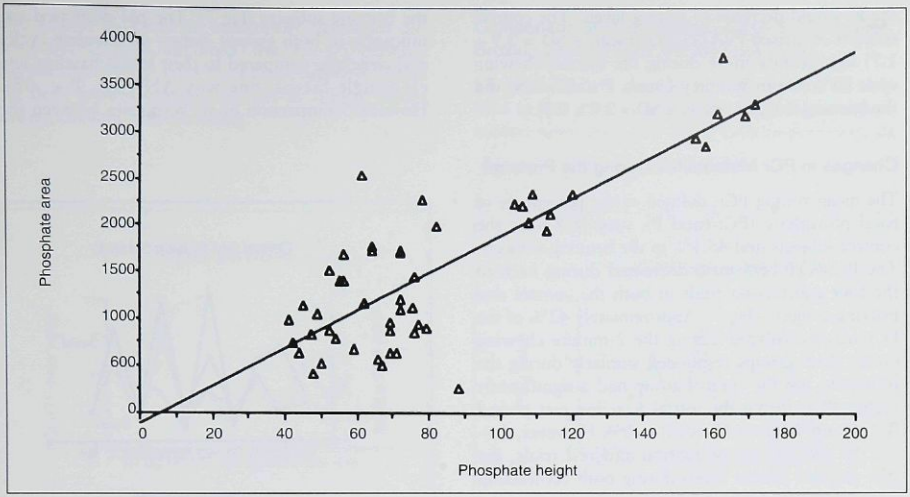

Fig 3 Linear regression analysis of the correlation between the area and height measurements of PCr and Pi from averaged spectra in each of the 10 trials in one subject. The Pearson's correlation coefficient of .83 indicates a strong association between the two measurements. Absolute values varied among subjects so that ratios of Pi and PCr were related to the stable ATP-beta peak and to each other ($y = 19.993x - 107.842$; $R^2 = .69$).

two-tailed, unpaired *t* test with the level of significance set at $P < .05$. The pH data were compared first among responses (ie, chewing) to the baseline levels using a single-factor one-way ANOVA with a Scheffe analysis at a level of significance set at $P < .05$. The pH data were then compared between the two groups of controls and bruxers for each response using a two-tailed, unpaired *t* test with level of significance at $P < .05$.

Data consisting of ratios of Pi/ATP beta, PCr/ATP beta, Pi/PCr were grouped for each of the 10 trials across six subjects in the control group and compared to the bruxing group in each trial using both measures of central tendency (ie, mean ± SD) and the nonparametric Mann-Whitney *U* test with the level of significance set at $P < .05$. Data were collected for similar responses providing six rest trials, three chewing trials, and one clenching trial to obtain the mean ± SD, and compared statistically by a single-factor one-way ANOVA with a Scheffe analysis at the level of significance of $P < .05$. The data were grouped for one response (ie, chewing) to compare the two experimental subgroups of bruxers with pain in the masseter muscle and bruxers without pain to the control group. Data were then compared within each of the three groups for a given response.

Studies by other investigators with repeated measures on separate days indicate that variability of the measurements as defined by the coefficient of variation (SD/mean × 100%) was minimal in the same subject compared to him/herself over 2 separate days and more varied among subjects.[32] The coefficient of variation for the 12 subjects during each of the measurements (ie, rest, chewing) ranged from 33.6% to 72.2% for Pi/ATP beta, 30.1% to 57.1% for PCr/ATP beta, and 17.1% to 39.2% for Pi/PCr.

Results

Changes in Pi Metabolites During the Protocol

The mean resting Pi calculated as the percentage of total phosphate (Pi/total P) was 6.4 ± 2.1% (mean ± SD) in both the control group and experimental subjects (ie, bruxers). The Pi/ATP beta increased in each of the four contraction trials of three chewing cycles and one clenching cycle in both groups (Fig 4). At the completion of each contraction trial,

the Pi would decrease to resting levels. The control subjects increased Pi/ATP beta (mean ± SD = 3.9 ± 1.7) significantly more during the second chewing cycle (W2; Mann-Whitney U test, $P < .05$) than did the bruxing subjects (mean ± SD = 2.7 ± 0.9).

Changes in PCr Metabolites During the Protocol

The mean resting PCr, defined as the percentage of total phosphate (PCr/total P), was 48.8% in the control subjects and 45.1% in the bruxing subjects. The PCr/ATP-beta ratio decreased during each of the four contraction trials in both the control and bruxing subjects (Fig 5). Approximately 42% of the PCr was hydrolyzed during the 2-minute chewing cycles. Both groups responded similarly during the protocols, but the control group had a significantly higher PCr during the initial baseline rest period (B2, Mann-Whitney U test, $P < .05$). However, during the succeeding contraction and rest trials, the PCr attained similar levels during both contraction and rest periods for both groups.

Changes in Pi/PCr Metabolites During the Protocol

The Pi/PCr ratio increased during each of the four contraction trials in both the control and bruxing subjects (Fig 6). Both groups responded similarly when the Pi/PCr ratio would decrease to the baseline levels during each of the rest periods and then increase during the three chewing and one clenching trials. However, the control group had a significantly higher Pi/PCr ratio during the third chewing cycle (W3, Mann-Whitney U test, $P < .05$) compared to the bruxing group.

Total Phosphate Levels

Nonbound phosphate comprising the areas of Pi, PCr, and three ATP peaks was compared as a ratio to ATP beta for all control subjects versus bruxing subjects. The control subjects had a significantly higher mean total phosphate level (12.5 ± 5.7%) compared to 10.5 ± 2.8% for the bruxing subjects (two-tailed, unpaired t test, $P < .01$). This occurred despite the larger standard deviation in the control data as compared to the bruxing subjects. The control subjects demonstrated about a 20% higher level of total phosphate ratio than the bruxing subjects.

Changes in pH During Exercise

The initial pH of the masseter muscle in the control subjects was 7.30 ± 0.01 (mean ± SD) and similar in the bruxing subjects (Fig 7). The pH decreased significantly in both groups during all chewing cycles and clenching compared to their initial baseline levels (single-factor, one-way ANOVA, $P < .05$). However, comparison of six responses between the

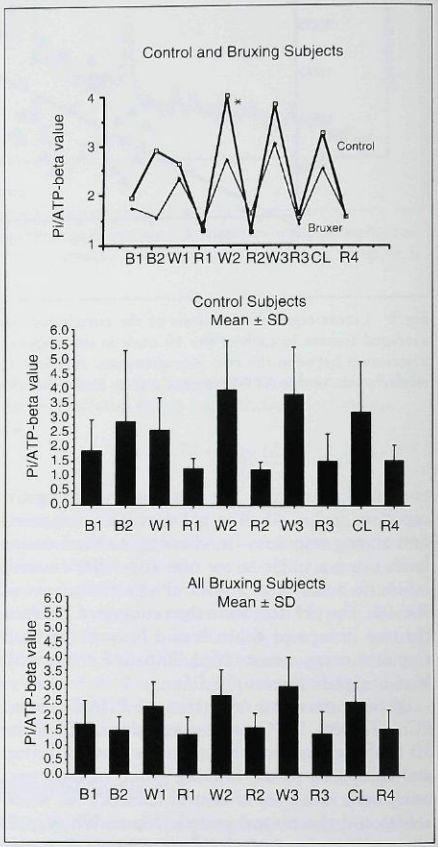

Fig 4 Mean and standard deviation of the Pi/ATP-beta values for the control subjects (n = 6) as a group are compared to that of the bruxers (n = 6) during 10 trials of chewing, resting, and clenching. B1 = first 2-minute baseline level, B2 = second 2-minute baseline level, W1 = chewing for 2 minutes, R1 = rest for 2 minutes, W2 = chewing for a second 2 minutes, R2 = rest, W3 = chewing for a third 2 minutes, R3 = rest, CL = clenching in the intercuspal position for 30 seconds, R4 = a final 2-minute rest. * = statistically significant difference between control and bruxing groups in second chewing trial (W2).

two groups indicated no statistically significant difference (two-tailed, unpaired t test, $P > .05$) between the controls and bruxers for a given response (Table 3). Both groups lowered the pH to similar values during chewing and clenching.

Comparison of Pi Between Control Subjects and Bruxing Groups With and Without Pain

The Pi/ATP-beta data were averaged for all subjects in one control group (n = 6) and two experimental subgroups (n = 3) for each response (ie, six

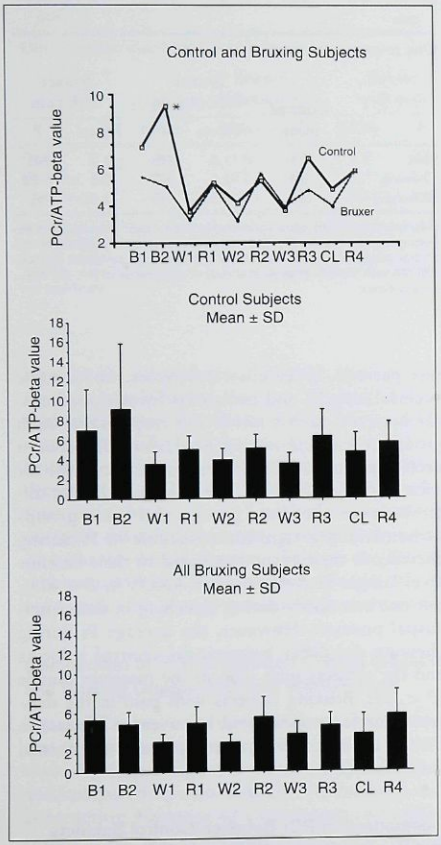

Fig 5 Mean and standard deviation of the PCr/ATP-beta values for the control subjects (n = 6) as a group are compared to that of the bruxers (n = 6) during 10 trials of chewing, resting, and clenching. B1 = first 2-minute baseline level, B2 = second 2-minute baseline level, W1 = chewing for 2 minutes, R1 = rest for 2 minutes, W2 = chewing for a second 2 minutes, R2 = rest, W3 = chewing for a third 2 minutes, R3 = rest, CL = clenching in the intercuspal position for 30 seconds, R4 = a final 2-minute rest. * = statistically significant difference between the control and bruxing groups in baseline trial (B2).

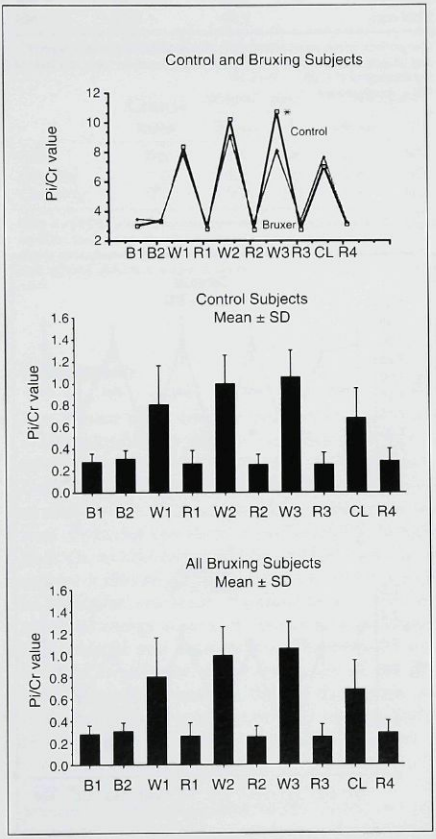

Fig 6 Mean and standard deviation of the Pi/PCr values for the control subjects (n = 6) as a group are compared to that of the bruxers (n = 6) during 10 trials of chewing, resting, and clenching. B1 = first 2-minute baseline level, B2 = second 2-minute baseline level, W1 = chewing for 2 minutes, R1 = rest for 2 minutes, W2 = chewing for a second 2 minutes, R2 = rest, W3 = chewing for a third 2 minutes, R3 = rest, CL = clenching in the intercuspal position for 30 seconds, R4 = a final 2-minute rest. * = statistically significant difference between the control and bruxing groups in third chewing trial (W3).

Table 3 Changes in pH

	Control subjects (n = 6)	Bruxing subjects (n = 6)	*P*
Baseline	7.28	7.29	NS
First chewing	7.03	6.93	NS
Second chewing	6.97	7.04	NS
Third chewing	7.02	6.97	NS
Clenching	7.09	7.09	NS
Final rest	7.30	7.32	NS

Comparison of the mean pH in each of six responses between control and bruxing subjects. *P* refers to a two-tailed, unpaired *t* test at a level of significance of $P < .05$.
NS = not significant.

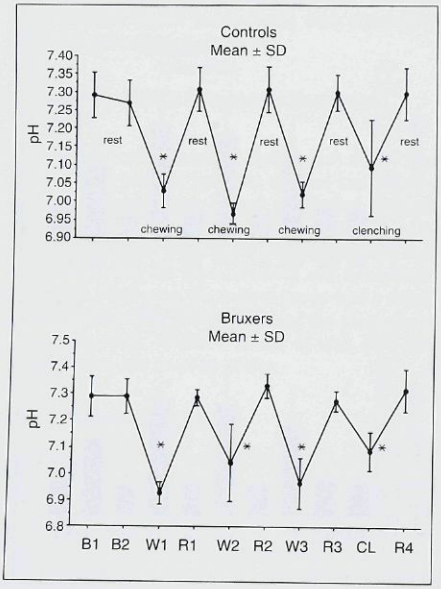

Fig 7 Mean and standard deviation of the pH values for the control subjects (n = 6) as a group are compared to that of the bruxers (n = 6) during 10 trials of chewing, resting, and clenching. B1 = first 2-minute baseline level, B2 = second 2-minute baseline level, W1 = chewing for 2 minutes, R1 = rest for 2 minutes, W2 = chewing for a second 2 minutes, R2 = rest, W3 = chewing for a third 2 minutes, R3 = rest, CL = clenching in the intercuspal position for 30 seconds, R4 = a final 2-minute rest. * = statistically significant difference between each chewing and clenching trial as compared to baseline.

Table 4 Pi/ATP-Beta Values

Three responses compared in one group

	Rest mean	Chewing Mean	Chewing *P*	Clenching Mean	Clenching *P*
Control	1.7	3.4	< .05	3.2	< .05
Bruxers without pain	1.5	2.8	< .05	2.5	NS
Bruxers with pain	1.5	2.5	< .05	2.6	NS

One response among three groups

	Control mean	Bruxer without pain Mean	Bruxer without pain *P*	Bruxer with pain Mean	Bruxer with pain *P*
Rest	1.7	1.5	NS	1.5	NS
Chewing	3.4	2.8	NS	2.5	< .05
Clenching	3.2	2.3	NS	2.6	NS

Data for Pi/ATP beta were compared between each response and the resting level for each group, then for each experimental subgroup to the control group for a given response. *P* refers to a single factor, one-way ANOVA with Scheffe analysis at a level of significance of $P < .05$. NS = not significant.

rest periods, three chewing cycles, one centric occlusal clench) and compared by two methods: *(1)* between each contraction response of each group to baseline resting level, and *(2)* between each experimental subgroup and the control for each response (Table 4). The resting Pi did not differ between the three groups. All three groups demonstrated a significant increase in Pi during chewing in their group compared to their baseline level (single-factor, one-way ANOVA, $P < .05$), but not necessarily during clenching in their intercuspal position. However, the average Pi during chewing did differ between the control subjects and the subjects with pain in the masseter muscle ($P < .05$). Bruxing subjects with pain in the masseter muscle demonstrated less average increase in their Pi during chewing as compared to the normal control subjects.

Comparison of PCr Between Control Subjects and Bruxing Groups With and Without Pain

The PCr/ATP-beta data were averaged for all subjects in one control group and two experimental subgroups for one response (ie, six rest periods, three chewing cycles, one intercuspal occlusal clench) and compared by two methods: *(1)* between each contraction response of each group to baseline resting level, and *(2)* between each experimental subgroup and the control for each response (Table 5). The resting PCr did differ

Table 5 PCr/ATP-Beta Values

Three responses compared in one group

	Rest	Chewing		Clenching	
	mean	Mean	*P*	Mean	*P*
Control	6.4	3.7	< .05	4.7	NS
Bruxers without pain	4.6	3.2	NS	3.3	NS
Bruxers with pain	5.8	3.3	< .05	4.3	NS

One response among three groups

	Control	Bruxer without pain		Bruxer with pain	
	mean	Mean	*P*	Mean	*P*
Rest	6.4	4.6	< .05	5.8	NS
Chewing	3.7	3.2	NS	3.3	NS
Clenching	4.7	3.3	NS	4.3	NS

Data for PCr/ATP beta were compared between each response and the resting level for each group, then for each experimental subgroup to the control group for a given response. *P* refers to a single factor, one-way ANOVA with Scheffe analysis at a level of significance of $P < .05$. NS = not significant.

Table 6 Pi/PCr Values

Three responses compared in one group

	Rest	Chewing		Clenching	
	mean	Mean	*P*	Mean	*P*
Control	0.3	1.0	< .05	0.7	< .05
Bruxers without pain	0.3	0.8	< .05	0.7	< .05
Bruxers with pain	0.3	0.8	< .05	0.8	< .05

One response among three groups

	Control	Bruxer without pain		Bruxer with pain	
	mean	Mean	*P*	Mean	*P*
Rest	0.3	0.3	NS	0.3	NS
Chewing	1.0	0.8	NS	0.8	NS
Clenching	0.7	0.7	NS	0.8	NS

Data for Pi/PCr were compared between each response and the resting level for each group, then for each experimental subgroup to the control group for a given response. *P* refers to a single factor, one-way ANOVA with Scheffe analysis at a level of significance of $P < .05$. NS = not significant.

between the control group and the bruxers without pain (single-factor, one-way ANOVA, $P < .05$) with the resting PCr lower in the bruxer group without pain. Two of the groups, the control and bruxers with pain, demonstrated a significant decrease in their PCr during chewing compared to their resting levels ($P < .05$), while the bruxers without pain did not demonstrate a statistically significant change during chewing. Because the bruxers without pain had a lower baseline PCr, the PCr did not significantly change during muscle contraction.

Comparison of Pi/PCr Between Control Subjects and Bruxing Groups With and Without Pain

The Pi/PCr data were averaged for all subjects in one group for one response (ie, six rest periods, three chewing cycles, one centric occlusal clench) and compared by two methods: *(1)* between each contraction response of each group to baseline resting level, and *(2)* between each experimental subgroup and the control for each response (Table 6). The resting Pi/PCr levels were the same in all three groups. Each group increased its Pi/PCr ratio during chewing and clenching as compared to its individual resting mean level (single-factor, one-way ANOVA, $P < .05$). In contrast, comparisons of the Pi/PCr levels among the three groups demonstrated no statistically significant differences: all three groups showed similar increases in the Pi/PCr ratios.

Discussion

The present study offers a perspective into how a craniomandibular muscle modifies its function when associated with an increase in its parafunctional time and with the associated factor of pain in that muscle. Our original hypotheses proposed that bruxing involves a sufficiently chronic increase in craniomandibular muscle function to require a change in the metabolism of the muscle. In particular, our study examined how the primary chemical energy source of adenosine triphosphate was utilized and replenished. The bruxers were equated to athletes in the frequency of use of a specific group of muscles. Pain in the muscle was considered to be a covariate that would modify this change in phosphate metabolism, resulting in a difference between the bruxers with pain and the bruxers without pain in the masseter muscle. Our previous electromyographic (EMG) studies of subjects with myofascial pain in the craniomandibular muscles had suggested that these muscles can be recruited with less activity in chewing and other functions, exhibit different coactivation patterns,[39–42] and, as other studies indicate, develop less occlusal force when pain is present.[43]

However, our data suggest events not predicted by the original hypotheses. The total phosphate level in the masseter muscle was significantly lower in the bruxing subjects than in the control subjects, an unexpected finding. The ^{31}P-NMR data from

highly trained athletes versus recreational athletes have suggested that the total phosphate is higher in the more highly trained individual in a muscle not primarily used in the athletic endeavor.[10,11,32,33] The data of Kurhanewicz et al[44] suggest that elite swimmers have a 75% higher resting phosphorus metabolite concentration than recreational athletes. Biopsies of muscle tissue in highly trained swimmers versus recreational athletes originally showed that the chemical concentrations of total phosphates and PCr were higher in the highly trained swimmers.[45-46] While the bruxing subject has a parafunctional habit that increases the use of that muscle, three possibilities exist that might explain the lowered total phosphate. First, the bruxing subject may increase the use of the masseter for bruxing but decrease the overall use of the muscle in other functions, including those that require greater force development, as in clenching and mastication. Second, the bruxing habit may be a low-force function that does not require metabolic adaptation to sustain and support it. Our six bruxing subjects demonstrated mild occlusal wear facets, suggesting that they were not heavy bruxers. Third, the bruxing subjects could be unique in having a genetically controlled lower phosphate level in the muscle.

The bruxing subjects developed less of an increase in inorganic phosphate (Pi) during chewing than the control subjects. When the bruxers were divided between those with and without pain in the masseter muscle, the bruxers with pain in the muscle demonstrated the least increase in Pi. Inorganic phosphate increases its concentration in muscle during contraction primarily due to the breakdown of a significant amount of ATP after it attaches to the contractile protein, myosin. Myosin functions as the enzyme ATPase, and it catalyzes the breakdown of ATP to adenosine diphosphate (ADP) and Pi. The Pi concentration increases as the myosin breaks down the ATP so that an indirect measure of the number of cross-bridges developed is reflected in the concentration of free Pi. Studies on human limb muscles by other investigators suggest that Pi increases to a steady state by 50 seconds with 30 contractions per minute of a biceps muscle.[32] Our data suggest that pain in the masseter muscle is associated with the less myosin-ATPase activity during chewing, and this finding correlates with our previous studies. Our previous data from 45 subjects with temporomandibular disorders, applying EMG to the masseter muscle, demonstrated that a significant number of the subjects with symptoms including pain in the head and neck muscles would decrease the amplitude of recruitment and probability of recruiting the masseter muscle.[39-42]

The phosphocreatine (PCr) levels within the masseter were significantly higher in the normal subjects prior to chewing and clenching than in the bruxing subjects without pain. Phosphocreatine is the first available chemical store used by muscle cells to replenish ATP, and it is chemically broken down or hydrolyzed to allow free phosphate to combine with ADP within the first few minutes of contraction.[16,17,47] The reaction of PCr $\longleftrightarrow$ Cr + Pi depends on sarcomere creatine kinase and myosin ATPase controlling the forward reaction and the mitochondrial creatine kinase and aerobic metabolism controlling the reverse reaction. Most reviews[14,15] of the creatine-phosphocreatine translocation emphasize its importance in mitochondrial respiration during increased muscle work. These studies have been done occasionally in human muscle using needle biopsies.[12-14] However, most studies employing muscle biopsies have used animals. Comparisons of PCr/ATP-beta ratios in frog sartorius muscles indicate that the ^{31}P-NMR evaluation renders a mean ratio of 6.74, while the chemical assessment is statistically similar at 8.14.[20] Both values are close to our mean baseline control values at 6.4. This would suggest that the bruxers had an abnormally low level of PCr.

The direct chemical measure suffers from the treatment of the muscle tissue with both freezing temperatures and then extraction of the chemicals to obtain the measurements, but it is the standard with which to compare. Studies in intact muscle and muscle extracts of PCr are similar, suggesting that PCr is almost completely free in the cytoplasm of the muscle fibers.[48] The muscle cell must then switch to alternative sources to make ATP after the initial use of PCr with the break down of glycogen, or anaerobic glycolysis, providing the next level. As muscle contraction continues, the blood supply increases in the working muscle, and sufficient oxygen and substrates of fatty acids and sugars become available as chemical substrates to the mitochondria to provide much more ATP.

Magnetic resonance studies of PCr have provided a more detailed assessment of changes in its concentration during dynamic function than the biochemical sampling approaches and have shown that PCr falls rapidly to a steady-state during contraction within the first 75 seconds.[32] Interestingly, studies on highly trained track athletes who have excelled to world standards demonstrate consistently higher levels of total phosphate, phosphocreatine, and inorganic phosphate in one of their muscles not primarily used in their sport, suggest-

ing a genetic basis for their fundamental differences from recreational athletes using the biceps muscle.[11] Resting PCr expressed as a percentage of the total phosphate is higher in elite swimmers (32.2%) than in recreational swimmers (25.7%) for the forearm flexor muscle.[44] The resting PCr of the forearm muscles of trained runners is 25% higher than that of control subjects.[11] Our data suggest that PCr, at significantly lower value in bruxers without pain, may be related to a genetic factor.

The ratio of Pi/PCr changed significantly as the subjects repeated the chewing over three trials. The control individuals increased this ratio with each succeeding trial, in contrast to the bruxers. The Pi/PCr ratio indirectly assesses the breakdown of ATP (ie, changes in Pi) and the three potential pathways which replenish ATP (ie, changes in PCr). An increase in the ratio indicates an imbalance in the use and replenishment of ATP with the myosin ATPase activity exceeding the PCr replacement of ATP. These data suggest two possibilities. First, the contraction of the masseter during chewing increasingly develops more cross-bridges and more force in the nonbruxing subjects than in the bruxing subjects. Second, PCr is needed less as the first source to replenish ATP in the nonbruxing subjects because the glycolytic and oxidative metabolic pathways are activated sooner than in the nonbruxing subjects. Our findings suggest that all three groups use a similar level of PCr and that the differences reside in the production of Pi.

The pH has been previously measured from muscle using invasive techniques with either a pH-sensitive microelectrode inserted directly into the cell, or an analysis of the distribution of the weak acids. Both approaches have inherent problems.[35,36] The ^{31}P-NMR provides an alternative method that is accurate, noninvasive, and effective in providing a continuous longitudinal assessment. Since Pi has a pH value close to neutrality, the intracellular pH markedly effects the relative amounts of $H_2PO_4^-$ and HPO_4^{2-}. The single spectrum for Pi exhibits a chemical shift (ie, parts per million) affected by the relative amounts of the two forms and a titration curve (parts per million versus pH) has been well developed for specific tissue such as the frog muscle at low temperatures.[49] The pH of various muscles assessed by the above three techniques suggest that mammalian muscles have a pH of around 7.4 to 7.5 at rest. Previous studies[32,36] of limb muscles have shown that pH falls during the transition from rest to exercise and usually within the first minute. The relatively small change is consistent with a minor contribution of glycolytic fibers compared with the more abundant oxidative fibers.

More than 70% of the human masseter muscle fibers are type I, which are slow to fatigue and contract.[22] The second highest proportion of muscle fibers in this muscle consist of the type IIB, which are rapidly fatiguing and fast-contracting fibers. In our study, both the control and bruxing groups demonstrated the same type of pH changes with a marked decrease in pH during chewing and clenching. The similar finding in both groups of subjects suggests that the masseter muscles have a similar composition of muscle fibers recruited during chewing and do not have one group of subjects using glycolytic fibers more than the other group.

Studies of the human forearm muscles (ie, wrist flexor muscles) have indicated that the Pi peak can demonstrate two peaks when work by the muscle exceeds 40% of the maximum strength developed by the muscle against a force transducer. This occurs only in a minority of untrained athletes, and not in professional runners.[24] The two peaks appear at a pH of 6.9 and at 5.9 to 6.4 in an arm muscle, which is considered relatively unused in untrained athletes. The authors suggest that in the lowest contraction levels of a muscle (ie, 20%), the type I and type IIA fibers are the fibers recruited. The type I fibers are slow-twitch red fibers rich in oxidative enzymes and poor in the concentration of glycolytic enzymes. The type IIA fibers are intermediate fast-twitch fibers with substantial amounts of both oxidative and glycolytic enzymes. Neither fiber develops much lactate while replenishing ATP, therefore the pH of the muscle changes minimally during contraction.[50,51] At higher levels of force development, the fast-twitch white fibers rich in glycolytic enzymes and low in oxidative enzymes are recruited and generate a second pool of Pi at a lower pH. This lower peak is lost with extension of the graded exercise beyond 18 minutes to 36 minutes. This suggests that the glycolytic fibers are exhausted by this time and that blood supply has increased within the muscle to remove lactate and replenish the oxidative pathways of the type I and IIA fibers. During recovery following the 18-minute tests, which evoked two Pi peaks, the Pi peak at 6.9 is lost while the peak at pH 5.9 to 6.4 remains for an additional 3 minutes. This differential recovery of the Pi peaks occurs because the Pi pool at pH 6.9 represents a fast regeneration of ATP and PCr in the oxidative type I and IIA fibers. In contrast, the persistent pool of Pi at pH 5.9 to 6.4 indicates the weak regeneration of the glycolytic fibers taking more time.

Our studies of the human masseter muscle did not demonstrate two pH peaks of Pi with the three 2-minute trials of chewing, suggesting that chewing

for this minimal time did not either recruit the type IIB fibers or the recruitment did not exhaust them. Correlative studies by our laboratory in the rabbit masseter using sustained 50-Hz stimulation to develop tetany over 40 minutes do not demonstrate two Pi peaks in this muscle. The rabbit masseter muscle has a similar composition to that of the human masseter (type I, IIA = 75%; type IIB = 25%), suggesting that the appearance of such a two-peaked Pi is difficult to evoke in this craniomandibular muscle.[52] The EMG recordings from the human masseter muscle indicate that mastication usually recruits the masseter muscle at moderately high intensity just below that of the maximum recruitment induced in this muscle, which is during intercuspal maximum clenching. Therefore, mastication should be sufficiently high to recruit the type IIB fibers. However, some of our parallel studies of the rabbit masseter, in which sustained electrical stimulation was applied to develop twitches (5 Hz), tetany (50 Hz), and assimilated tetany (50 Hz on for 500 milliseconds and off for 500 milliseconds), indicate that the muscle is able to recover its changes in Pi and PCr more effectively during the cyclic tetany than sustained tetany, and with fewer pH changes. These rabbit studies suggest that despite mastication recruiting the masseter at relatively high EMG levels, its pattern of muscle contraction with some intercontraction rest periods provides a mechanism for the more fatigue–sensitive fibers to remain active.

Interestingly, Park et al[24] cite some experimental data that suggest two Pi peaks occur in a number of pathologic conditions: dialysis patients complaining of muscle weakness; canine muscular dystrophy; and the hypoxic and ischemic condition of the dog and cat brains. These data suggest that the Pi shifts with two peaks could be good indicators of change in muscle function associated with fibers that are unable to metabolically maintain the muscle function. Such a question remains to be studied in subjects with muscle pain. Our preliminary study of three bruxing subjects with pain does not suggest a differential recruitment of muscle fibers in brief uses of the muscle but might with more sustained contraction of the muscle.

Disadvantages of this study reside in not obtaining an independent measure of muscle force, which, for craniomandibular muscles, can be best determined in the intact human by occlusal force measurements.[26,27] Chewing was standardized by having all subjects chew the same type of bolus, of the same consistency, and on the same side. Ideally, the trials should have consisted of sustained activity sufficient to maximally recruit the muscles near fatigue, but discomfort to the patients excluded this approach. Our pilot work has shown that normal healthy subjects evoke pain with sustained maximum and submaximum clenching for even 2 minutes, which supports previous studies. Sustained chewing for a 30-minute trial would have been preferable but too uncomfortable for our control subjects and patients with masseter pain. Consequently, we chose a relatively brief, reproducible test of the masseter without using a nonmetallic occlusal force transducer. Our baseline values of Pi/PCr (0.30 ± 0.10) are higher than those found in other muscles with 0.10 in the human flexor digitorum profundus,[53] and 0.12 in the wrist flexors.[10] In Lam and Hannam's study[26] of the human masseter, the Pi/PCr resting values varied with the region and were highest in the masseter region just anterior to the condyle (0.70 ± 0.17) and similar in the anterior border region of the muscle (0.40 ± 0.13) and the posterior border of the muscle (0.42 ± 0.11). This suggests that the human masseter has a resting baseline Pi/PCr value higher than the limb muscles. These three regions then increased their Pi/PCr values to 1.09 to 1.70 (depending on the region) during clenching on either molar. Our Pi/PCr values increased from a baseline of 0.30 to 0.88 during chewing and slightly lower to 0.70 during intercuspal clenching. Our lower values for Pi/PCr may reflect partial saturation of our signal, as well as chewing using the masseter differently than sustained clenching.

The potential to analyze subjects with craniomandibular disorders using ^{31}P-NMR is still unexplored, but it has been utilized as a valuable technique to assess the effect of changes in circulation and impairment of that circulation on muscle and other organ functions.[54] Additionally, ^{31}P-NMR has been applied to studies of muscles during fatigue[55] and in pain.[28] The potential application of ^{31}P-NMR to determine if interplay occurs among factors involved with fatigue, altered circulation, and pain in the muscle is a promising avenue of new research in TMD.

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Resumen

Espectroscopia de Resonancia Magnética del Músculo Masetero Humano en Personas con Bruxismo y en Personas Asintomáticas

Se evaluaron los músculos maseteros de seis personas asintomáticas (cinco hombres, una mujer) y seis personas que sufrían de bruxismo (cuatro hombres, dos mujeres), durante la masticación, por medio de espectroscopia de resonancia magnética (^{31}P-NMR). Los espectros NMR fueron coleccionados en un imán de cuerpo entero GE Sigma 1.5T con un espiral de superficie ^{31}p/^{1}H afinado doblemente. Se completaron tres pruebas de descanso-masticación-descanso, de dos minutos de duración. En cada prueba se reunieron los promedios de los espectros de fosfato inorgánico, fosfocreatina, y tres picos de ATP. Las personas que bruxaban presentaban una concentración menor de fosfato total y fosfocreatina en comparación a los sujetos asintomáticos en descanso. Las personas que bruxaban aumentaron el nivel de fosfato inorgánico durante la masticación, pero el aumento fue significativamente menor que en el caso de los controles. Los niveles de pH durante el descanso y la masticación fueron similares tanto en los controles como en las personas que bruxaban. Estos resultados preliminares indican que las personas que bruxan presentan un metabolismo de fosfato alterado durante el descanso y presentan un patrón de metabolismo de fosfato diferente durante la masticación en comparación con los sujetos asintomáticos.

Zusammenfassung

Magnetresonanzspektroskopie des menschlichen M. masseter bei Bruxern und asymptomatischen Personen.

Die Mm. masseteres von sechs asymptomatischen Personen (fünf Männer, eine Frau) und sechs Bruxern (vier Männer, zwei Frauen) wurde während des Kauens durch Magnetresonanzspektroskopie (^{31}P-NMR) untersucht. Die NMR-Spektren wurden mit einem GE Sigma 1.5 T Ganzkörpermagneten mit einer zweifach abgestimmten ^{31}P/^{1}H Oberflächenspule aufgezeichnet. Zweiminütige Registrierungen mit posturaler Lage/Kauen/posturaler Lage wurden dreimal wiederholt. Für jede Messung wurde das durchschnittliche Spektrum von anorganischen Phosphaten und Phosphokreatin berechnet und drei ATP-Peaks aufgezeichnet. Bruxer wiesen bei posturaler Unterkieferlage eine kleinere Konzentration des Totalphosphats und des Phosphokreatins auf als asymptomatische Personen. Bei Bruxern erhöhte sich das anorganisches Phosphat während des Kauens signifikant weniger als bei den Kontrollpersonen. Die pH-Werte während der posturalen Unterkieferlage und während des Kauens waren bei beiden Gruppen ähnlich. Diese vorläufigen Resultate weisen darauf hin, dass Bruxer einen veränderten Phosphatmetabolismus während der Ruhelage und ein anderes Phosphatmetabolismusmuster während des Kauens als die asymptomatischen Personen zeigen.