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Individuality of masticatory performance and of masticatory muscle temporal parameters

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ABSTRACT

Objective: Mammalian mastication serves to improve intra-oral food reduction. Insufficient food reduction creates potential swallowing problems, whereas over-reduction may accelerate tooth wear and increase feeding time. Either extreme has consequences. The study's objectives were: (1) to study the relationship between food reduction, number of chews in a sequence, and chewing rate, (2) to study how controlling the number of chews and chewing rate variability affects food reduction, and (3) to assess how dentoskeletal morphological and electromyographical (EMG) characteristics impact food reduction.

Design: Twenty-three healthy, fully-dentate adults chewed a standardized test food under three conditions: (1) no control, (2) number of chews controlled, and (3) number of chews and chewing rate controlled. EMG activity was sampled from masseter and temporalis muscles bilaterally. Demographic, occlusal contact area in maximum intercuspation, and cephalometric data were obtained.

Results: In uncontrolled conditions, food reduction and bout duration varied more than expected across subjects. Subjects with poor reduction under controlled conditions were those with poor reduction under uncontrolled conditions. Only occlusal contact area correlated with chewing performance under uncontrolled conditions. Chewing cycle duration, EMG burst duration, and EMG peak onset latency increased when the number of chews was restricted. EMG amplitude, a surrogate for bite force, increased in tasks controlling the number of chews and chewing rate. Chewing rate variability was difficult to diminish below individual-specific levels.

Conclusions: Results: provided evidence that bite force, chewing rate, chewing performance and chewing bout duration reflected individual preferences. Future work will determine whether similar findings occur among other mammals.

1. Introduction

Mastication is the process whereby food particles are reduced in size and mixed with saliva to facilitate safe passage through the oropharynx. Several measures have been developed to quantify the rate of food particle size reduction. Chewing efficiency was originally defined as the ability to grind a given portion of test food within a set time, and chewing time (or chewing performance) as the time period necessary to grind and swallow a defined portion of test food (Helkimo, Carlsson, & Helkimo, 1978; Laurell & Lundgren, 1985; Owens, Buschang, Throckmorton, Palmer, & English, 2002). More recently, chewing efficiency has been defined as the number of chewing cycles required to attain a particle size half the initial size, and chewing performance as the median particle size (X_{50}) attained after a given number of chews (Olthoff, van der Bilt, Bosman, & Kleizen, 1984; van den Braber, van der Glas, van der Bilt, & Bosman, 2001). The Rosin-Rammler equation (Olthoff et al., 1984), an industry standard originally developed for quantifying particle size distribution in geologic studies, is often used to

Abbreviations: A_{MI}, occlusal contact area in maximum intercuspation; ANB, angle formed between point A nasion and point B; ANS, anterior nasal spine; Co, condylion; Co-Gn, distance between condylion and gnathion; CV, coefficient of variation; EMG, electromyography; FMA, angle formed by Frankfort horizontal (line segment defined by porion and orbitale) and mandibular plane (line segment defined by Go and Me); Go, gonion; Go-Gn, distance between gonion and gnathion; Gn, gnathion; Me, menton; MI, maximum intercuspation; N, depending on context either nasion or slope of linear portion of Rosin-Rammler equation solution; N_C, number of chews in a trial; O, orbitale; P, porion; PVS, polyvinyl siloxane; RMS1, normalized root mean square amplitude of first EMG burst in a trial; RMSΩ, normalized root mean square amplitude of final EMG burst in a trial; S, sella; SD, standard deviation; SNA, angle formed between sella nasion and point A; SNB, angle formed between sella nasion and point B; SN-GoGn, angle formed by line segment defined by sella and nasion and line segment defined by gonion and gnathion; T_B, EMG burst duration or time between onset and offset of an EMG burst; T_C, natural mean chewing cycle duration; T_P, EMG peak onset latency or time from EMG burst onset to time of peak activity; UAFH/LAFH, ratio of upper anterior face height (distance between nasion and point A) and lower anterior face height (distance between point A and menton); X_{50}, median particle size

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quantify X_{50} (Eberhard et al., 2012; Hirano, Hirano, & Hayakawa, 2004; Olthoff et al., 1984). Another method for studying chewing performance involves using two-colored gum and assessing the degree of color mixing after a given number of chews (Hayakawa, Watanabe, Hirano, Nagao, & Seki, 1998; Liedberg & Owall, 1995).

Studies of chewing performance and chewing efficiency generally define the 'chew unit' as a chewing stroke (Olthoff et al., 1984), gape cycle (Palmer, Rudin, Lara, & Crompton, 1992; Smith, 1992) or chewing cycle (Ross, Eckhardt et al., 2007; Sanchez-Ayala, Farias-Neto, Campanha, & Garcia, 2013). These terms are often used interchangeably, with the unit typically defined either from a maximum jaw opening to the next maximum jaw opening or from one maximum jaw close to the next maximum jaw close (Ross, Eckhardt et al., 2007). Alternatively, it may be defined from onset to onset or peak to peak of a masticatory muscle electromyographic (EMG) burst, e.g., (Gerstner & Goldberg, 1991), or from motoneuron bursting patterns in fictive animal preparations, cf. (Barlow, Lund, Estep, & Kolta, 2010).

Evidence suggests that dentoskeletal morphological and occlusal surface area traits (Laird, Vogel, & Pontzer, 2016) along with certain demographics (Lund & Kolta, 2006) play roles in chewing efficiency. Many studies have reported relationships between occlusal surface area and chewing efficiency or performance (Laird et al., 2016; Luke & Lucas, 1985; Magalhaes, Pereira, Marques, & Gameiro, 2010; Owens et al., 2002; Wilding, 1993). Subjects with specific skeletal malocclusions seem to have poorer performance than those without such malocclusions (van den Braber et al., 2001). Although numerous studies have discussed age-related changes in mastication (Chavez & Ship, 2000; Gerstner, Madhavan, & Braun, 2014; Heath, 1982; Miura et al., 2000; Peyron, Woda, Bourdiol, & Hennequin, 2017), very few if any have identified definitive gender differences (Ferrario & Sforza, 1996; Gerstner & Parekh, 1997; Gonzalez, Sifre, Benedito, & Nogues, 2002).

Several masticatory jaw movement parameters may be associated with variation in masticatory performance; however, results vary from study to study. One study reported that poorer performers had increased cycle-to-cycle variability, longer jaw opening duration, larger excursive movements, and increased lateral jaw movement velocity compared with better performers (Lepley, Throckmorton, Parker, & Buschang, 2010). However, other evidence suggests that increasing the lateral or excursive movement of the jaw improves efficiency (Yamashita, Hatch, & Rugh, 1999). Some chewing pattern variation likely reflects adaptive, complex interplays between dentoskeletal morphology and the physical properties of the food (Yamashita et al., 1999). That is, some cycle-to-cycle variability reflects ongoing adjustments to current food properties to facilitate food particle size reduction (Lund & Kolta, 2006; Lund, 1991; Ross, Dharia et al., 2007). On the other hand, there is evidence that chewing performance varies apparently according to individual preference, with some chewers simply swallowing larger particles than others, regardless of food properties, i.e., some are "fast swallowers" whereas others are "slow swallowers" (Engelen, Fontijn-Tekamp, & Van Der Bilt, 2005). Whatever the case, these findings suggest that experimental removal of or control over variability could impact chewing performance. The role of experimental control, specifically control of chewing rate and number of chews, in chewing performance is investigated in this study.

Under routine conditions, one major factor that likely influences X_{50} is the number of chews, i.e., the more chews one performs on a given mouthful, the finer the particles. But, for mammals subject to natural selection pressures, there are ecological drawbacks to increasing the number of chews. For one, increasing the number of chews increases the duration of feeding sequences, and this can impact the total daily activity budget allotted to feeding (Ross, Washington et al., 2009). Increasing the number of chews also increases dental wear (Estebaranz, Galbany, Martinez, & Perez-Perez, 2007; Lucas & Omar, 2012; Mahoney, 2006; Solounias, Fortelius, & Freeman, 1994; Wetselaar, Vermaire, Visscher, Lobbezoo, & Schuller, 2016). Tooth wear has been linked to increased mortality (Kojola, Helle, Huhta, & Niva, 1998; Tyler, 1986; Veiberg et al., 2007) and decreased fecundity (King et al., 2005; Wright, King, Baden, & Jernvall, 2008) in certain mammals. Additionally, bite force is likely to play an important role in performance (Engelen et al., 2005; Marquezin, Kobayashi, Montes, Gaviao, & Castelo, 2013; Pereira, Duarte Gaviao, & Van Der Bilt, 2006); but, bite force also increases tooth wear (Diracoglu et al., 2011; Johansson, Kiliaridis, Haraldson, Omar, & Carlsson, 1993; Kiliaridis, Johansson, Haraldson, Omar, & Carlsson, 1995); however, cf. (Cosme, Baldisserotto, Canabarro, & Shinkai, 2005). Thus, increasing bite force or the number of chews in order to improve chewing performance is likely to have long-term consequences.

There is evidence suggesting that a relatively narrow particle size range exists in the pre-swallowing bolus, whereas the number of chews, chewing sequence duration and muscle activity can be more variable across subjects (Peyron et al., 2017); however, this is not a universal finding, cf. (Engelen et al., 2005). If a narrow particle size range exists, this would suggest that a person with traits associated with poorer performance would likely either chew more or modify muscle activity patterns, e.g., increase bite force (Engelen et al., 2005), in order to achieve a 'target' level of food reduction necessary for swallowing.

On the other hand, there is also evidence that an individual who lacks sufficient occlusion or who has lost teeth necessary to reduce food effectively will swallow larger particles and thus run the risk of problems with swallowing (Feldman, Kapur, Alman, & Chauncey, 1980). It seems logical that individuals with traits that reduce masticatory ability would either change the temporal architecture of feeding in order to compensate for the 'maladaptive' traits, e.g., increase the time spent feeding or the number of chews per mouthful, or such individuals would manifest impacts similar to those seen in the elderly suffering tooth loss, e.g., eat soft or less nutritious foods, reduce food intake, swallow relatively larger particles (Feldman et al., 1980). However, evidence linking impaired masticatory ability and nutrition is surprisingly weak (N'Gom P & Woda, 2002).

Several important questions thus remain unanswered. First, what is the range of X_{50} seen in the healthy population, and what factors are associated with this range? Does occlusal contact area or skeletal morphology predict performance or chewing architecture among such a group? If not, is there evidence that individuals adjust chewing rate, the timing of EMG parameters, bite force, or chewing rate variation to achieve better performance?

These questions and issues are addressed in this study. The study sought to determine whether there was a relatively similar level of chewing performance among a healthy population, and if so, what aspects of chewing architecture appeared to be modulated across individuals to achieve a similar performance range. Assuming that variation in chewing sequences and timing would occur, we also assessed whether there were demographic, morphologic, occlusal, or muscle activity patterns that could account for variation in chewing sequences and timing.

Importantly, evidence suggests that a given person's swallowing threshold is due more to food properties than oral physiological factors (Engelen et al., 2005). Indeed, the elastic and plastic rheological properties of test foods can significantly impact a number of masticatory features, e.g., chewing rate, muscle activity, sensory perception (Foster, Woda, & Peyron, 2006). For this reason, this study used an artificial test food whose physical properties we attempted to control carefully, recognizing the consequential problems and limitations associated with using a test food that cannot be swallowed (Foster et al., 2006).

2. Materials and methods

2.1. Subjects

A sample of 23 healthy, fully dentate young-adult subjects was involved in the study (see Table 1 for demographics). Subjects' rights

Table 1
Descriptive statistics of subject sample[a].

Gender	Age	Ht	Wt	A_{MI}	T_C	BPM	FMA	ANB	SNA	SNB	SN-GoGn	Co-Gn	Go-Gn	UAFH/LAFH
F	24	172	59.0	122.4	751.9	80	28.4	4.8	82.3	77.5	35.7	124.5	89.7	79.9
F	24	160	62.6	105.4	819.7	73	29.8	1.8	81.7	79.9	33.9	130.5	90.4	66.4
F	21	155	45.4	61.8	917.4	66	14.2	−1.1	80.3	81.4	19.1	122.6	90.6	77.1
F	21	155	59.9	62.1	862.1	70	21.6	1.9	76.2	74.3	28.1	110.2	80.0	90.6
F	27	167	67.1	91.9	833.3	72	28.3	−1.5	80.2	81.7	30.3	122.8	89.9	78.7
F	24	157	57.6	132.7	885.0	68	31.7	3.6	78.1	74.5	40.2	112.2	83.8	80.1
F	19	160	54.4	54.3	751.9	80	24.4	1.7	82.3	80.6	30.2	126.5	85.9	79.1
F	21	157	49.9	45.9	833.3	72	24.2	3.4	79.4	76.0	36.8	125.4	78.9	83.5
[b]F	22	167	68.0	104.4	769.2	78	26.0	1.0	80.4	79.4	30.8	137.2	90.2	79.6
F	18	163	56.7	86.1	675.7	89	20.1	−0.2	79.1	79.2	29.8	124.6	88.4	86.2
F	26	175	62.6	67.0	740.7	81	16.3	2.7	82.1	79.4	21.2	109.1	80.8	85.7
[c]F	20	167	58.9	92.6	649.4	92	9.2	−0.8	80.9	81.7	14.4	138.0	95.8	91.9
F	23	170	58.9	1.4	598.8	100	14.3	2.4	83.5	81.1	24.7	128.1	93.3	86.0
F	19	170	59.8	65.8	1,063.8	56	26.9	1.5	73.1	71.6	35.6	130.5	94.7	79.3
M	25	188	88.0	87.9	900.9	66	20.6	0.3	85.5	85.2	24.0	116.3	80.3	81.0
M	24	170	65.8	39.2	961.5	62	28.5	5.1	83.0	77.9	35.1	131.1	90.9	80.5
M	22	185	83.9	22.7	751.9	80	28.8	1.2	84.0	82.8	29.0	143.5	86.6	61.4
M	23	183	83.9	82.3	714.3	84	19.2	−1.2	78.3	79.6	27.3	123.4	87.9	79.8
M	24	183	74.8	35.3	877.2	68	26.3	0.4	79.8	79.3	34.7	119.6	76.7	76.6
M	22	180	78.0	42.5	885.0	68	27.6	−1.7	76.8	78.4	30.2	129.4	88.9	88.8
[d]M	28	180	74.8	61.2	787.4	76	20.3	4.6	85.2	80.7	23.8	117.8	77.5	78.0
M	32	185	86.2	108.0	970.9	62	22.1	−0.1	79.5	79.5	31.9	140.6	92.7	83.7
[e]M	30	195	86.2	91.8	598.8	100	12.1	−0.5	83.4	83.9	18.3	131.9	85.5	95.7
Mean	23.4	171.5	67.1	72.4	808.7	76	22.6	1.3	80.6	79.4	28.9	125.9	86.9	81.3
S.D.	3.5	11.6	12.5	32.7	119.1	12	6.2	2.1	3.0	3.1	6.6	9.2	5.6	7.5

[a] Key: Ht, height in cm; Wt, weight in kg; A_{MI}, occlusal contact area in maximum intercuspation in mm^2; T_C, natural mean chewing cycle duration determined from gum chewing in ms; BPM, metronome rate (beats per minute). Remaining variables in table are cephalometric variables; refer to Fig. 1 for landmark identifications. FMA, angle formed by Frankfort horizontal (line segment defined by porion and orbitale) and mandibular plane (line segment defined by Go and Me); ANB, angle formed between point A, nasion, and point B; SNA, angle formed between sella, nasion and point A; SNB, angle formed between sella, nasion and point B; SN-GoGn, angle formed by line segment defined by sella and nasion and line segment defined by gonion and gnathion; Co-Gn, distance between condylion and gnathion; Go-Gn, distance between gonion and gnathion; UAFH/LAFH, ratio of upper anterior face height (distance between nasion and point A) and lower anterior face height (distance between point A and menton).

[b] Test food data not analyzed from Task 2 in this subject.

[c] Test food data not analyzed from Task 2 and 3 for this subject.

[d] Test food data not analyzed from all three tasks for this subject.

[e] Test food and masseter muscle data not analyzed from all three tasks for this subject.

were protected by the University of Michigan's medical IRB, and written informed consent was obtained from all subjects. Screening and many experimental procedures have been described elsewhere (Fulks, Callaghan, Tewksbury, & Gerstner, 2017); such procedures will be briefly described, below. Subjects meeting the following inclusion criteria were consented and involved in the study: (1) no chewing side preference, (2) no chewing difficulties, (3) no gum chewing habit, (4) no orthodontic work within the previous year, (5) no temporomandibular disorders (TMD), as defined by RDC-TMD criteria (Dworkin & LeResche, 1992), (6) no musculoskeletal, gastrointestinal, nor neurological conditions, (7) no use of medications known to have oral motor side effects, (8) no history of eating disorders, (9) no recent radiation exposure. Inclusion criteria, confirmed by oral examination, were Angle's Class I molar relationship, and presence of full dentition, less third molars.

2.2. Cephalometrics

Landmarks on the skull and mandible were identified on lateral cephalographs of the subjects, and the landmark positions were digitized twice by an orthodontist (Fig. 1) (Dolphin Imaging, v.11.7, Dolphin Imaging and Management Solutions, Chatsworth, CA). Mean landmark values from the two digitizations were used to construct standard clinical and custom measurements (see Fig. 1 and Table 1).

2.3. Surface electromyography (EMG) signal recording and filtering

EMG data from superficial masseter and anterior temporalis muscles bilaterally (Fig. 1) were measured with bipolar surface electrodes (Ag/AgCl, 1.8-m snap-on leads, MVAP Medical Supplies, Newbury Park, CA). A ground electrode was placed over the left mastoid process. Digitized data (1 kHz sampling rate, Octal Bioamp, PowerLab 8/35, LabChart Pro v. 8.0.4, ADInstruments, Colorado Springs, CO) were band-pass filtered (20–500 Hz), notch-filtered (60 Hz), full-wave rectified, and smoothed with a moving average window set to 5% of the sampling rate, cf. (Ives & Wigglesworth, 2003).

2.4. Test food

Test food tablets (CutterSil Putty Plus, Universal Plus Hardener, Heraeus Kulzer, South Bend, IN) were created using a standardized template (Plexiglas, 4.76-mm thickness, 12-mm diameter), allowed to set for 1 h, trimmed to remove flash, and weighed. Only tablets weighing 0.85 g + 0.05 g were used; all others were discarded. Careful controls of mixing ratios, set time, time to tablet use, and tablet size were undertaken to minimize the impact of variation in physical properties of test tablets on results (Foster, Woda, & Peyron, 2006).

2.5. Experimental procedure

2.5.1. Mean chewing rate calculation

Subjects chewed gum (Trident Original, Mondelez International, Deerfield, IL) until softened. Subsequently, a sequence of > 30 consecutive chews, without intervening talking or swallowing, was identified and used to calculate a mean chewing cycle duration (T_C) for each subject. This subject-specific mean T_C was used to set the metronome

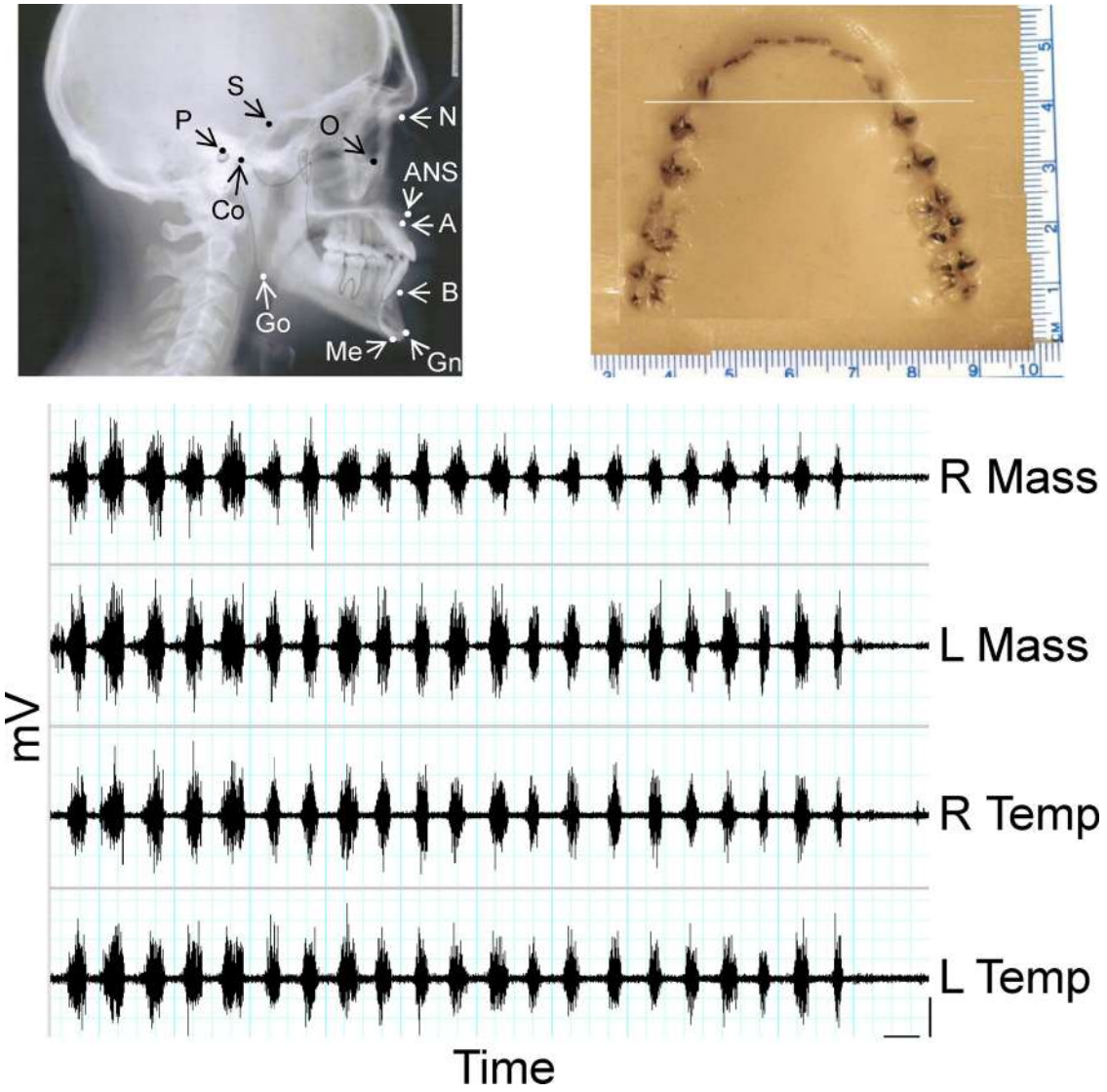

Fig. 1. Top left. Example of a lateral cephalograph. Key: A, point A; ANS, anterior nasal spine; B, point B; Co, condylion; Go, gonion; Gn, gnathion; Me, menton; N, nasion; O, orbitale; P, porion; S, sella. Top right. Example of a bite registration. Horizontal line distinguishes anterior contacts from posterior contacts; only posterior contacts were analyzed. Bottom. Example of EMG data from a trial representing the ($-N_C$,$-T_C$) task. EMG traces, top to bottom, are right masseter, left masseter, right temporalis, left temporalis. In this case, the trial consisted of 21 chewing cycles. Bars, lower right, represent 1000 ms (abscissa) and 0.2 mV (ordinate).

rate used in Task 3 (see below).

2.5.2. Blocks, trials and tasks

While seated comfortably, subjects performed three tasks distinguished by the following instructions: Task 1 − "Chew as naturally as possible until the food is ready to swallow. Then spit out all particles into the cup." Task 2 − "Chew as naturally as possible, but chew ten times only; that is, only bite down ten times. Then spit out all particles into the cup." Task 3 − "Chew to the beat of the metronome and only chew ten times; that is, bite down ten times to the beat of the metronome. Then spit out all particles into the cup." Task 1 included trials in which neither T_C nor number of chews (N_C) was controlled and will be referred to as ($-N_C$,$-T_C$). Task 2 controlled N_C, but not T_C and will be referred to as ($+N_C$,$-T_C$). Task 3 controlled both N_C and T_C and will be referred to as ($+N_C$,$+T_C$). For Task 3, the metronome rate used was the subject-specific mean T_C described in the previous section.

During an experiment, subjects performed five blocks, with each of the three tasks being performed once per block. 'Trial' will refer to a single replicate of a task. One tablet was chewed per trial. The order of tasks was randomized within each block. No instructions were given regarding chewing side. A cup of water was supplied to use as necessary to rinse and remove all particles between trials. After rinsing, the liquid and particles were spat into the cup assigned to the trial. Trials not conforming to all of the above specifications were redone. By the end, each subject had chewed 15 tablets.

2.6. EMG analysis

EMG burst onsets and offsets occurred when EMG voltages went above and below, respectively, a threshold voltage set 10% above the baseline voltage. For Task 1 (see Section 2.5.2), the number of EMG bursts in a trial defined the number of chews, N_C, for that trial. For the other two tasks, we verified that only ten EMG bursts occurred per trial. If more or fewer than ten EMG bursts occurred, such trials were discarded.

For all three tasks, the time between two successive EMG burst onsets defined chewing cycle duration (T_C). EMG burst duration (T_B), and peak amplitude onset latency (T_P) were calculated for each chewing cycle in a trial. Within-trial variation in T_C, T_B, and T_P was defined by the coefficient of variation (CV) = standard deviation/mean

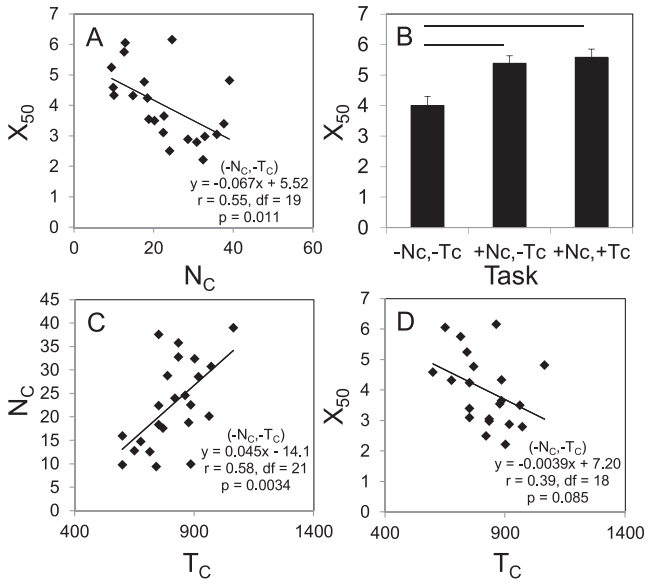

Fig. 2. A. Scatterplot of mean number of chews in a trial (N_C) vs. median particle size X_{50} for the uncontrolled trials (-N_C,-T_C) across subjects. B. Mean X_{50} for the three tasks. Error bars are 1 SD. Horizontal bars show significance levels $p < 0.001$. C. Scatterplot of T_C (in ms) vs. N_C for the (-N_C,-T_C) trials. D. Scatterplot of T_C vs. X_{50} for the (-N_C,-T_C) trials.

for each muscle and trial. EMG amplitude can be used to estimate bite force, e.g., (Park, McCall, & Chung, 2012; Stepp, 2012), with root mean square (RMS) being a popular choice for estimating force (Park et al., 2012; Stepp, 2012). The RMS of rectified EMG bursts was calculated for the first (RMS1) and final (RMSΩ) EMG bursts in a trial using an algorithm in LabChart Pro v. 8.0.4, (ADInstruments, Colorado Springs, CO). For each task and muscle, these RMS values were expressed as proportions of the maximum RMS value obtained from the respective muscle for the given subject across trials and tasks. The EMG measurements of T_C, T_B, T_P, and RMS were then averaged across left and right muscle pairs as well as across the trials constituting a given task.

2.7. Test food particle treatment and analysis

Test food particles from each trial were kept in separate cups. Contents of each cup were washed, disinfected, left to dry for 24 h, and separated through a series of seven sieves with mesh sizes of 5.6, 4.0, 2.8, 2.0, 0.85, 0.425, and 0.25 mm (U.S.A. Standard Test Sieves, Hogentogler & Co., Inc., Columbia, MD). Sieves were shaken for two minutes to separate particles by size (Gilson Company, Inc., Lewis Center, OH). Contents found on each sieve were weighed to the nearest 0.01 g.

Chewing performance was measured using the Rosin-Rammler equation, $Q_w = 100[1\text{-}2_{50}^{-(D/X)N}]$ (Rosin & Rammler, 1933), where Q_w was the weight percentage of particles passing through a sieve size smaller than D (in mm), X_{50} was median particle size (in mm), and N was the slope of the linear part of the fitted equation (Olthoff et al., 1984). A relatively small X_{50} indicated relatively good performance. Calculations were made using an algorithm in MatLab (Brezani & Zelenak, 2010). X_{50} was averaged across trials within tasks for each subject.

2.8. Wax bites and occlusion analysis

Subjects were asked to bite in maximum intercuspation (MI) on bite registration wax (Hygenic Yellow Bite wax–Coltene/Whaledent). The wax registrations were photographed from a distance of 1 m against a dark background to provide high-contrast areas representing tooth contacts. Millimeter rulers placed in-plane with the wax were used for calibration (Fig. 1).

Eight-bit versions of wax images were used with a color threshold of 90 to digitize total premolar and molar bite contact areas (ImageJ software, NIH, Bethesda, MD). Two researchers independently calculated contact areas, which were averaged together for analysis. Inter-rater reliability of this technique was considered acceptable (Pearson's product moment, $r^2 = 0.997$; slope = 1.02; intercept = −0.21).

2.9. Statistical procedures

General linear mixed models with repeated measures were used, with subject treated as a random effect, task as a repeated measure, and all continuous variables as covariate fixed effects (SPSS, v.24, Chicago, IL). Pairwise comparisons from models reaching statistical significance were made with a Bonferroni correction. Multiple linear regression models, using the stepwise procedure, were performed to identify variable subsets that best fit variation in performance. The resultant beta coefficients were evaluated for significance. Tolerance values were also obtained to evaluate collinearity among variables in the model. Variables were removed from models when tolerance < 0.2. A paired *t*-test was used for certain *pre-hoc* tests involving within-subject, between-two-task comparisons. Correlation analyses were performed in Excel (MS Office 10, Ver. 14). A $p < 0.05$ defined the level of significance in all *pre hoc* tests.

3. Results

Table 1 lists descriptive statistics of the subjects individually, with sample means and 1 standard deviation (SD) also provided at the bottom of the table. Listed are demographics, occlusal contact area, natural chewing rate, and cephalometric data. The table indicates four subjects for whom some data were not included in analyses (see footnotes in table). For the three tasks, an average of 96% of the original test food weight was recovered, and there were no significant differences between tasks in this regard ($F[1,22] = 1.359$, $p = 0.256$).

3.1. Relationship between performance, number of chewing cycles and chewing rate

Fig. 2A plots the number of chews, N_C, against X_{50} for the uncontrolled, (-N_C,-T_C) task by subject. Two things are noteworthy. First, X_{50} had a relatively large range from ~2–6 mm across subjects. This provided evidence against the hypothesis that a similar level of chewing performance exists across healthy subjects. Second, the range of N_C (6–39 chews) coupled with the significant relationship between N_C and X_{50}, (see Fig. 2A, lower right of plot), suggests that poorer performers could have improved performance by increasing the number of chews; however, this did not happen. This provides further evidence against the existence of a 'target' level of chewing performance shared across healthy subjects.

Fig. 2B plots the mean (1 SD) X_{50} for the three tasks. X_{50} differed significantly across tasks ($F[2,23] = 21.235$, $p < 0.001$). Pairwise comparisons revealed that X_{50} for the (-N_C,-T_C) task was significantly less than the X_{50} for the other two tasks ($p < 0.001$); no significant difference existed between the (+N_C,-T_C) and the (+N_C,+T_C) task. Fig. 2C plots T_C against N_C for the uncontrolled (-N_C,-T_C) task. The plot shows that subjects who chewed more (greater N_C) also had slower 'natural' chewing rates (longer duration T_C). Thus, slower chewers had longer chewing sequences, and this was due to both a slower chewing rate and to an increased number of chews per trial. However, a stepwise linear regression revealed that N_C was a better predictor of X_{50} than was T_C for this task. Fig. 2D provides an additional demonstration of this, in that the relationship between T_C and X_{50} was not significant for the (-N_C,-T_C) task. Nevertheless, a trend for those with slower chewing rates to have improved performance is evident. Thus, these results provide evidence that individual variation in performance was due mainly to

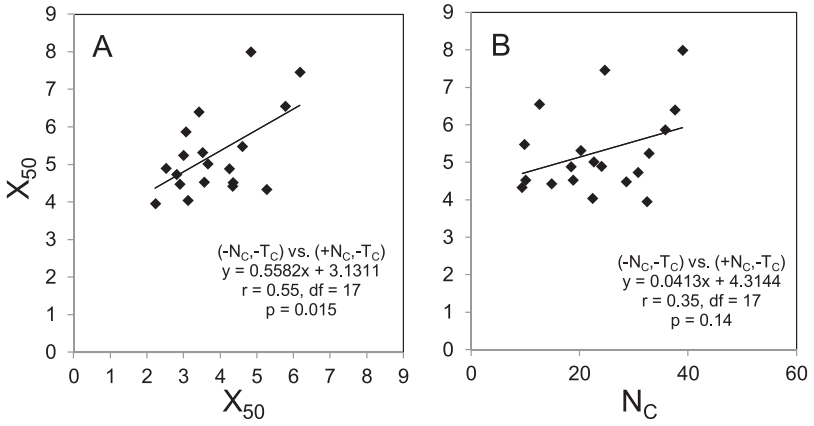

Fig. 3. A. Correlation between X_{50} for the ($-N_C$,$-T_C$) vs. ($+N_C$,$-T_C$) task. B. Correlation between N_C used by subjects in ($-N_C$,$-T_C$) trials vs. the X_{50} achieved for ($+N_C$,$-T_C$) tasks.

longer chewing bouts, and to a lesser degree to slower chewing rates.

3.2. Comparison of performance under controlled and uncontrolled N_C conditions

Fig. 3A compares the X_{50} between the ($-N_C$,$-T_C$) and ($+N_C$,$-T_C$) tasks across subjects. The significant correlation indicates that subjects with poorer performance when N_C was controlled were also those subjects with poorer performance when they were allowed to chew until the food was swallowable.

To assess whether subjects with poorer performance under N_C-controlled conditions attempted to improve their performance by increasing N_C in the uncontrolled condition, we compared the N_C in the ($-N_C$,$-T_C$) with X_{50} in the ($+N_C$,$-T_C$) tasks (Fig. 3B). This correlation was not significant, although a trend is evident. Since the controlled condition served to make performance on a per-chew basis comparable between subjects, these results indicate that relatively poorer performers did not elect to improve performance by chewing more when permitted to do so.

3.3. Relationship between performance and morphological and EMG characteristics

We posited that people possessing mechanically unfavorable traits, e.g., long faces, smaller occlusal contact area, shorter EMG bursts, would use more chews to improve performance when engaged in the uncontrolled task. If this did not occur, we hypothesized that they would be the subjects manifesting poorer performance (larger X_{50}). To test this, we did stepwise linear regression analyses to determine whether variation in any demographic, morphologic, EMG or occlusal traits would account for variation in X_{50} or N_C in the ($-N_C$,$-T_C$) tasks. Results indicated that A_{MI} was significantly negatively correlated with X_{50} ($F[1,19] = 4.94$, $p = 0.039$), with 20.6% of the variation in performance being accountable for in A_{MI}. That is, subjects with greater occlusal contact area had better performance. No other variables predicted X_{50} or N (Table 2).

We also tested this hypothesis by using results for the ($+N_C$,$-T_C$) task, which allowed us to assess variation in X_{50} when N_C was controlled. Stepwise linear regression models indicated that no demographic data (gender, age), cephalometric, occlusal contact area nor EMG variables predicted X_{50} in these tasks (Table 2).

Finally, we tested the hypothesis that subjects might increase bite force to compensate for experimental control over number of chews and chewing rate variability. Fig. 4 shows mean (1SD) RMS values of the first and last temporalis EMG burst in trials representing each of the three tasks; similar results were obtained for the masseter muscle. Significant differences existed across tasks for the initial bite (masseter

Table 2
Data associated with variables excluded from step-wise regressions.

[a]DV; Task	[a]IV	[c]Beta	t	p	Tolerance
X_{50}; $-N_C$,$-T_C$					
	T_C (T)	−0.115	−0.552	0.588	0.997
	T_B (T)	0.051	0.244	0.810	0.992
	T_P (T)	0.179	0.862	0.400	0.982
	Age	0.074	0.344	0.735	0.942
	Ht	0.071	0.336	0.741	0.974
	Wt	0.082	0.390	0.701	1.000
	FMA	0.114	0.535	0.599	0.962
	ANB	0.270	1.351	0.193	0.999
	SNA	−0.073	−0.343	0.736	0.956
	SNB	−0.291	−1.441	0.167	0.968
	SNGoGn	0.135	0.638	0.532	0.963
	CoGn	−0.086	−0.409	0.687	0.992
	GoGn	−0.210	−1.010	0.326	0.967
	UAFH/LAFH	−0.042	−0.198	0.845	0.999
	RMS1 (M)	0.258	1.272	0.220	0.985
	RMSΩ (M)	0.244	1.159	0.262	0.928
	RMS1 (T)	0.031	0.147	0.885	0.971
	RMSΩ (T)	−0.186	−0.754	0.461	0.701
[b]N; $-N_C$,$-T_C$					
	T_C (T)	0.006	0.965	0.355	0.684
	Age	−0.299	−1.120	0.286	0.551
	Ht	0.019	0.195	0.849	0.348
	A_{MI}	−0.071	−2.086	0.061	0.301
	SNB	−0.820	−2.354	0.038	0.451
	CoGn	−0.099	−1.213	0.251	0.665
	UAFH/LAFH	−0.058	−0.452	0.660	0.482
	RMS1 (T)	−5.797	−0.653	0.527	0.467
	RMSΩ (T)	−44.495	−2.705	0.020	0.242
[b]X_{50}; $+N_C$,$-T_C$					
	T_C (T)	0.001	0.475	0.645	0.370
	Wt	0.028	1.134	0.218	0.937
	A_{MI}	−0.011	−1.487	0.168	0.892
	ANG	−0.232	−1.517	0.160	0.529
	CoGn	−0.035	−0.895	0.392	0.463
	UAFH/LAFH	−0.139	−2.497	0.032	0.534
	RMS1 (T)	3.457	1.077	0.307	0.368
	RMSΩ (T)	5.444	1.210	0.254	0.364

[a] DV, dependent variables; IV, independent variables (see text for variable abbreviations).

[b] SPSS does not provide results on excluded variables from step-wise regressions when no variables are entered into the equation; therefore, these reported results were obtained using the all variables entered method. Note, using the entered method did not lead to statistically significant results.

[c] Beta for the stepwise model is "beta in"; for the all variables entered method it is the unstandardized coefficient (SPSS nomenclature).

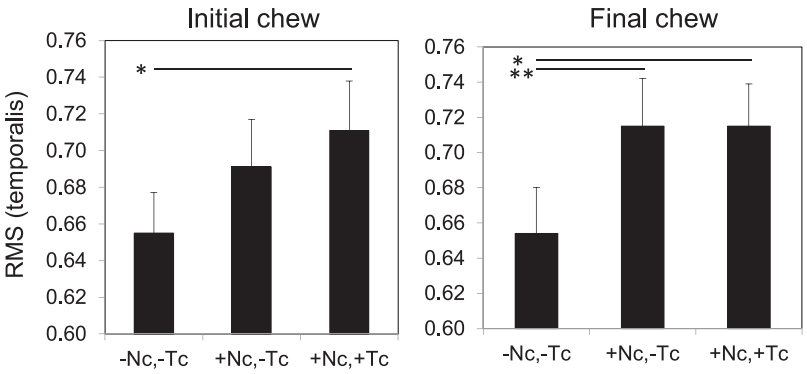

Fig. 4. RMS of temporalis EMG bursts representing the initial (RMS1, left) and final (RMSΩ, right) chews in trials representing the three tasks. RMS is expressed as a proportion of the maximum RMS obtained for each subject during the experiment (see Section 2.6). Results were similar for the masseter muscle. Horizontal bars identify pairwise significant differences; * = $p < 0.02$; ** = $p < 0.001$.

results, F[2,20] = 7.4, p = 0.004; temporalis results, F[2,21] = 4.9, p = 0.018) and final bite (masseter results, F[2,20] = 0.72, p = 0.004; temporalis results, F[2,21] = 6.6, p = 0.006). Pairwise comparisons revealed that EMG RMS values for the first chew were significantly increased for the ($+N_C,+T_C$) compared with the ($-N_C,-T_C$) task. For the final chew, RMS values were significantly increased for both the ($+N_C,-T_C$) and ($+N_C,+T_C$) tasks compared with the ($-N_C,-T_C$) task. These results suggest that the metronome significantly increased bite force throughout entire trials, whereas only controlling number of chews led to slightly increased bite forces initially, which increased significantly as such trials progressed towards completion.

3.4. Relationship between individuality, within-trial chewing rate variability, and chewing performance

Fig. 5A plots the mean (1 SD) within-trial coefficients of variation in chewing cycle duration $CV(T_C)$ for the ($+N_C,-T_C$) and ($+N_C,+T_C$) tasks. (We elected to compare these two tasks only, because CV calculations were based upon equal sample sizes for this comparison.) The $CV(T_C)$ for ($+N_C,-T_C$) trials was significantly greater than the $CV(T_C)$ for ($+N_C,+T_C$) trials (paired t = 2.16, df = 21, p = 0.042), indicating that the metronome had an impact on chewing rate variability.

However, considerable chewing rate variation occurred even with the metronome. Fig. 5B compares within-trial $CV(T_C)$ for ($+N_C,-T_C$) versus ($+N_C,+T_C$) tasks by subject. Interestingly, note that subjects with relatively high variation in T_C when T_C was *not* controlled maintained a relatively high variation in T_C when chewing to the beat of the metronome. This suggests that subjects with inherently high chewing rate variability had more difficulty keeping pace with the metronome than did those subjects with inherently low chewing rate variability. It also provides support for the hypothesis that chewing rate variability is individual-specific.

We hypothesized that variation in chewing rate during a trial would reflect a subject's ability to adapt to variation in food bolus properties; therefore, increased variation in chewing rate, i.e., a relatively high CV (T_C), should reflect improved performance. Fig. 6A compares the CV (T_C) with X_{50} for the ($+N_C,-T_C$) tasks, and the results provide evidence against the hypothesis that variation in T_C positively impacted performance. On the other hand, Fig. 6B compares the X_{50} for ($+N_C,-T_C$) tasks with X_{50} for ($+N_C,+T_C$) tasks, and this plot indicates that performance was similar within subjects, regardless of the presence or absence of the metronome. The relationship was highly significant (results in bottom right of plot). It is important to note that without the most apparent outlier in Fig. 6B, the relationship remained significant (r = 0.69, df = 16, p = 0.00153). Furthermore, there were two additional outliers, which became apparent when the one outlier was removed; with these removed, the relationship remained significant (r = 0.55, df = 14, p = 0.027). Thus, the results shown in Fig. 6 suggest that chewing performance was individual-specific, but that individual differences in chewing rate variability played little role in variation in performance.

3.5. Influence of experimental controls on EMG timing parameters

Fig. 7 shows how EMG timing variables were altered by controlling N_C and T_C. The columns, left to right, show results for chewing cycle duration (T_C), EMG burst duration (T_B), and EMG peak onset latency (T_P). Fig. 7A–C shows results for comparisons of means for the temporalis muscles (similar results were obtained for the masseter). Addition of experimental control affected T_C as measured using temporalis EMG bursts (F[2,38] = 8.257, p = 0.001). Surprisingly, Fig. 7A shows that controlling N_C alone increased T_C significantly.

Fig. 7B shows that T_B differed significantly across tasks (F[2,17] = 16.63, $p < 0.001$), with all pairwise differences being significant. T_B was the longest for the ($+N_C,-T_C$) task and shortest for the ($-N_C,-T_C$) task, with the ($+N_C,+T_C$) task being intermediate. Thus, controlling N_C increased EMG burst durations, whereas controlling both N_C and T_C decreased EMG burst durations towards the 'natural' mean duration.

Similarly, Fig. 7C shows that T_P differed significantly across tasks (F

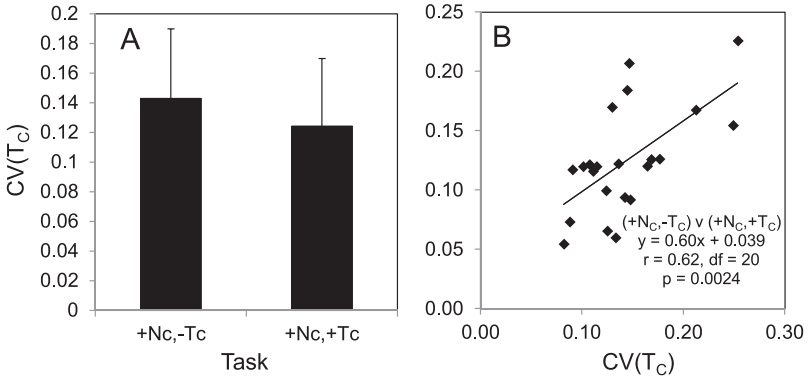

Fig. 5. A. Mean $CV(T_C)$ for ($+N_C,-T_C$) vs. ($+N_C,+T_C$) tasks. B. Correlation comparing $CV(T_C)$ for ($+N_C,-T_C$) vs. ($+N_C,+T_C$) tasks by subject.

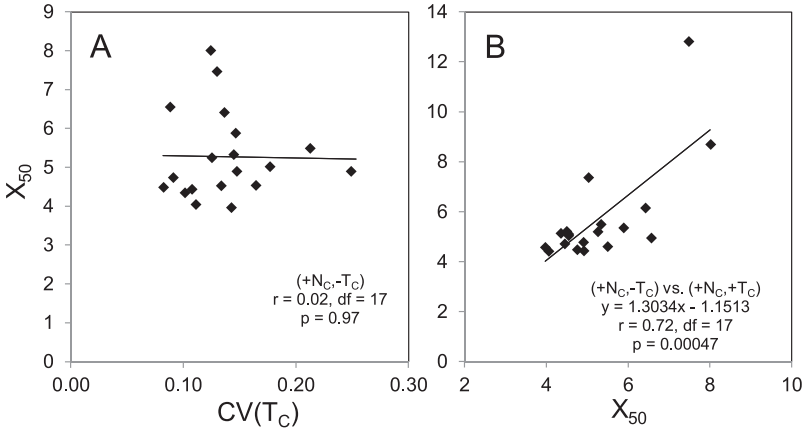

Fig. 6. A. Within-trial CV(T_C) plotted against X_{50} for the ($+N_C$,-T_C) task. B. X_{50} for the ($+N_C$,-T_C) vs. ($+N_C$,$+T_C$) tasks.

[2,31] = 7.99, p = 0.002), with T_P being significantly longer for the ($+N_C$,-T_C) task compared with the other two tasks. Peak onset latencies for both (-N_C,-T_C) and ($+N_C$,$+T_C$) tasks had similarly short durations. Thus, controlling number of chews increased T_P, whereas controlling both number of chews *and* chewing rate returned the T_P to values similar to those of the uncontrolled task condition.

Fig. 7D–F shows results for comparisons of the CV for T_C, T_B, and T_P for the temporalis muscle (again, similar results were obtained for the masseter). Significant differences were found within all analyses ($p < 0.003$). We anticipated that CV would be significantly impacted by the metronome; however, the results show that controlling N_C alone significantly reduced CV. The CV for all three EMG variables and for both muscles was significantly higher for the (-N_C,-T_C) tasks than for the other two tasks (Fig. 7D–F). The only additional significant pairwise comparison was between the ($+N_C$,-T_C) and ($+N_C$,$+T_C$) tasks for the temporalis CV(T_C) (Fig. 7D), which was not seen for the masseter.

4. Discussion

The results provided evidence that performance varied significantly among individual subjects. This finding differs from previous results (Peyron, Mishellany, & Woda, 2004) where inter-individual variability in food particle sizes was minimal, indicating a similar performance level across individuals. However, the previous study used actual foods and recovered between 40% and 80% of the original food weights, whereas this study had subjects chew a commonly-used test food made of polyvinylsiloxane (PVS) and recovered about 96% of the original tablets. The difference in recovery percentages between the two studies is likely due to the edibility or palatability of the foods. That is, subjects masticating actual foods are probably more likely to swallow some accidentally (which would impact the measured median particle size), whereas subjects eating an artificial test food are less likely to do so. Indeed, a key limitation of using artificial test foods is due to their impact on swallowing (Foster et al., 2006). Also, as subjects may vary in their aversion to swallowing test foods, they may also vary in their aversion to chewing them to swallowable size, which may have impacted variation in median particle sizes among our subjects.

Whatever the case, it is important to recognize that our findings for individual preferences in performance support other previous work (Engelen et al., 2005). Specifically, previous investigators found that the number of chews to swallowing was strongly correlated across vastly different foods for individual subjects, suggesting an individual preference for chewing bout lengths (Engelen et al., 2005). Although we controlled chewing bout lengths in two of our tasks, our results in Figs. 3A and 6B seem to demonstrate individual preferences in particle size. It is probable that particle size preference and chewing bout length preference are necessarily correlated and complimentary.

Indeed, results in Fig. 2A show that subjects who chewed more (greater N_C) in the uncontrolled tasks ended up with smaller X_{50} (better performance), suggesting that if individuals would have had similar

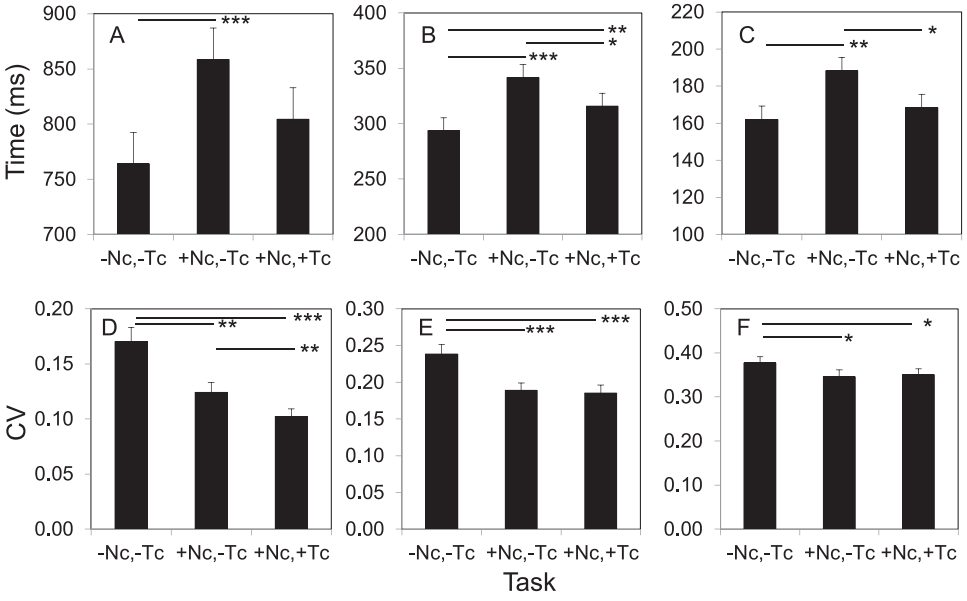

Fig. 7. Mean (1 SD) EMG timing variables (top) and CV for the variables (bottom) across the three tasks. Temporalis muscle results are shown; masseter muscle results were similar. Columns are T_C (left), T_B (middle), and T_P (right). Ordinate is time in ms (top) or unitless (bottom). Horizontal bars depict significant pair-wise comparisons, with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

numbers of chews during uncontrolled trials, performance could potentially have been more similar across subjects. And yet, this did not occur, suggesting individual preferences for chewing bout length, particle size or both.

One surprise was that subjects who had more chewing cycles (greater N_C) in the uncontrolled ($-N_C$,$-T_C$) trials also had slower chewing rates (increased T_C) (Fig. 2C). We anticipated that faster chewing would reduce the rate of food reduction, and therefore reduce performance, on a per-chew basis due either to the inverse relationship between force and velocity in muscle (Hill, 1938) or to the inverse relationship between speed and accuracy (Fitts, 1954). These inverse relationships are only partially born out in results; Fig. 2D showed only a trend for those with slower chewing rates to have better performance.

In actuality, those subjects with the smallest X_{50} used a combination of slower chewing rate (Fig. 2D) and longer chewing bouts (Fig. 2A) to improve performance; by contrast, those subjects characterized by larger X_{50} not only chewed more rapidly, but had shorter chewing bouts. This suggests that some subjects may be more 'thorough' or 'deliberate' chewers, employing both slower chewing rates and longer chewing bouts to achieve improved performance. This is reminiscent of previous results where subjects varied greatly in terms of the number of chews to swallow, independent of food properties or salivary flow rate (Engelen et al., 2005). The suggestion is that time to swallow may be an individual-specific 'target' parameter, and that chewing rate and chewing bout length are parameters modified by individuals to achieve this target. Future studies should determine whether and what factors interact during development to create this pairing of chewing rate and chewing bout length.

Subjects with poorer performance in the uncontrolled ($-N_C$,$-T_C$) trials were those with poorer performance in the controlled ($+N_C$,$-T_C$) trials (Fig. 3A), again supporting the idea that some subjects prefer not to chew as long as others (Engelen et al., 2005). Put another way, subjects who were relatively poor performers as determined under controlled N_C, appeared to use no compensatory mechanisms to improve their performance when given the opportunity to do so. In one sense, this variation in performance is not surprising given the fact that chewing occurs intra-orally; hence, individuals have virtually no way of comparing performance with peers. Thus, performance is a private experience, based on personal assessment of performance.

We posited that variation in performance would likely be due in part to variation in morphological and occlusal traits, and EMG parameters among the subjects. The results suggested that variation in morphological traits and most EMG variables played little role, whereas occlusal contact area play an important role in performance. This corroborates results in some previous investigations (English, Buschang, & Throckmorton, 2002; Magalhaes et al., 2010; Owens et al., 2002), but not all (Toro, Buschang, Throckmorton, & Roldan, 2005).

Bite force plays an important role in performance (Engelen et al., 2005; Marquezin et al., 2013; Pereira et al., 2006), and subjects in our study appeared to increase bite force when experimental controls over number of chews and chewing rate variation were imposed (Fig. 4). However, our surrogate for bite force (EMG RMS values) did not appear to be related significantly to variation in performance, at least as determined using step-wise linear regression analysis. This suggests that subjects manipulated bite force as a means of compensating for experimental control; however, the compensation was just sufficient to meet individual preferences in performance.

There is evidence that people with highly variable chewing kinematics have poorer performance (Lepley et al., 2010). Chewing rate, in particular, appears to be relatively invariant in humans (Bhatka, Throckmorton, Wintergerst, Hutchins, & Buschang, 2004), other primates (Ross et al., 2010) and many other mammals (Gerstner & Gerstein, 2008). Nevertheless, variation in within-sequence chewing rate occurs, and this variability likely reflects adaptations to changes in food physical properties (Reed & Ross, 2010). We posited that increased variation in chewing rate would be associated with better performance (relatively smaller X_{50}). Although chewing rate variation did not appear to impact performance (Figs. 2B, 6A), it is noteworthy that the metronome seemed relatively ineffective at removing chewing rate variability. In fact, each subject reduced the variability proportionate to their 'natural' variability (Fig. 5B), and each subject had similar performances with and without the metronome (Fig. 6B). Thus, chewing rate variability appeared to be individual specific, yet unrelated to performance across subjects. Further work will be required in order to determine whether chewing rate variability plays a role in performance *within* individuals, and to confirm that chewing rate variability is an aspect of kinematics that does not impact performance as much as other kinematic parameters do (Lepley et al., 2010).

Although chewing rate variability was only slightly reduced by the metronome (Fig. 5), EMG timing variables were more profoundly affected by experimental control (Fig. 7). Surprisingly, controlling the number of chews alone was sufficient for subjects to manifest increased cycle duration (Fig. 7A), with concomitant increases in EMG burst durations (T_B, Fig. 7B) and time to peak onset (T_P, Fig. 7C). Because subjects were restricted to ten chews in these trials, we hypothesize that they increased T_B and T_P in order to increase the duty cycle of mastication, thereby attempting to improve performance in the face of the imposed experimental restriction.

On the other hand, the control of cycle duration variability by the metronome in the ($+N_C$,$+T_C$) task reduced the duration of EMG variables such that their values returned towards the uncontrolled durations (Fig. 7A–C). Thus, one of the metronome's most noticeable effects seemed to have been to reduce the duration of the duty cycle. This being the case, we would have expected a significant impact on performance manifest in the ($+N_C$,$+T_C$) task. However, this was not the case, as seen in Fig. 2B. The similar performance levels for both the ($+N_C$,$-T_C$) and ($+N_C$,$+T_C$) tasks suggest a ceiling effect due mainly to restriction on number of chews. A systematic, chew-by-chew evaluation of performance could provide insights in this regard.

One aspect of Fig. 7A requires some explanation. If the ($+N_C$,$+T_C$) trials were performed to a metronome set at the subjects' natural chewing rates, why were the ($-N_C$,$-T_C$) rates significantly faster than the ($+N_C$,$+T_C$) rates? Recall that the metronome rates were determined from subjects' chewing gum. The differences between chewing rates during the ($-N_C$,$-T_C$) and ($+N_C$,$+T_C$) trials, therefore, reflect differences between the rates occurring during test-food chewing versus gum chewing, respectively. The implication is that chewing rates associated with gum chewing must have been significantly different from chewing rates during reduction of this test food, an implication supported by previous work (Foster et al., 2006). Gum chewing is often used as a 'test food' for studies of masticatory function in humans (Anastassiadou & Heath, 2001; Gerstner & Fehrman, 1999; Gerstner et al., 2014; Gerstner, Marchi, & Haerian, 1999; Hada, Tabe, Tsuka, Yamauchi, & Muneoka, 1977; Hayasaki et al., 2003; Liedberg & Owall, 1995; Prinz, 2004); however, the results in Fig. 7A suggest that further investigations comparing gum chewing to the chewing of actual food stuffs is in order. We would hypothesize that the difference is likely to lie in the fact that a chewing sequence involving food reduction produces recognizable variation in chewing cycle durations as food consistency changes (Nakamura & Katakura, 1995; Schwartz, Enomoto, Valiquette, & Lund, 1989). Such variation probably does not exist when chewing gum. Thus, differences between the mean gum-chewing rates, which were used to derive chewing rates in the ($+N_C$,$+T_C$) tasks versus the mean chewing rates calculated from the ($-N_C$,$-T_C$) tasks may reflect these differences.

Figs. 5A and 7D–F demonstrate the impact of the metronome on intra-trial variation in EMG parameters. There was a decrease in the variability in T_C associated with the metronome, which reached significance in the pairwise comparison (Fig. 5A) and within the temporalis data in the linear models involving all three tasks (Fig. 7D). Interestingly, variation in the intra-EMG burst variables (Fig. 7E, F) did not show similar reductions in variation. This suggests that when

subjects attempted to synchronize T_C to the metronome, T_B and T_P remained relatively decoupled from this entrainment. We believe this provides evidence that the duty cycle of chewing can respond to physical properties of food on a chew by chew basis, independent of the ongoing chewing rhythm (T_C). Put another way, natural chewing may occur at a rhythmic rate, which is sufficiently slow to allow EMG activity to perform work tailored to the current food bolus properties. Indeed, there exists evidence that chewing at rates faster than the natural rate reduces performance possibly as a result of insufficient time for feedback to play a role in modulating the current chewing cycle (Fulks et al., 2017).

The results raise several interesting insights into the motor control of chewing. It is well established that mammalian chewing rates are relatively invariant, varying by 10–20% around a relatively fixed mean value (Gintof, Konow, Ross, & Sanford, 2010; Ross, Eckhardt et al., 2007), where the mean value defines a given species' natural chewing rate (Druzinsky, 1993; Gerstner & Gerstein, 2008; Ross, Reed et al., 2009). During the ($-N_C$,$-T_C$) task, the $CV(T_C)$ was about 17.0%, as measured using temporalis EMG data (Fig. 7D); this falls within the range of previous reports on mammalian chewing rate variation (Gintof et al., 2010; Ross, Eckhardt et al., 2007). However, the fact that chewing rate variability remained about 12.4% when chewing to the metronome beat (Fig. 5A), and that it seemed to be individual-specific (Fig. 5B) suggests that 12.4% might approximate a minimal degree of variability inherent in masticatory circuitry *in vivo*.

With regards to the ($+N_C$,$-T_C$) task, it was surprising that simply controlling the number of chews, without the deliberate modification of chewing rate, lead to a significant reduction in chewing rate (Fig. 7A) and chewing rate variability (Fig. 7D). Our interpretation of this is that, the act of mentally counting the number of chews, which was an intrinsic component of this task (see Section 2.5.2), likely significantly modified the central control of chewing that operates under routine conditions (Lund & Kolta, 2006; Nakamura & Katakura, 1995).

Finally, several study limitations should be described. First, our subjects represented a relatively homogenous sample of young healthy adults. It is likely that younger populations (Gerstner et al., 2014), older populations (Karlsson, Persson, & Carlsson, 1991; Kohyama, Mioche, & Bourdiol, 2003), populations with significant tooth wear or tooth loss (Hotta et al., 2000; van der Bilt et al., 1993), or with dentoskeletal malocclusions (Magalhaes et al., 2010; Owens et al., 2002; van den Braber et al., 2001) would show different results from those reported. Nevertheless, the study provides an important baseline for future comparisons.

Second, the study lacked a post-study feedback or debriefing period, during which time we could have identified what the subjects actually thought during experiments or how they interpreted commands. This may have been helpful in understanding subjects' understanding of "chewing until swallowable", whether they thought the test food was easy or difficult to chew, etc. Sensory testing (feedback to assess food physical properties) has been shown to contain useful information relevant to chewing performance (Foster et al., 2006), and inclusion of such testing in future studies will be important. Third, although we were careful to mix, form and weigh the test food tablets, we were unable to confirm the physical properties of each tablet, e.g., elastic modulus, toughness. Variation in physical properties may have contributed to 'noise' in the performance data, cf. (Foster et al., 2006; Peyron, Lassauzay, & Woda, 2002), thus impacting some results. However, mixing procedures were carefully controlled, and tablets were used within a specific time window after mixing. Finally, it is important to recognize that these results pertain to findings from a study limited to human subjects. Clearly, morphology and occlusal characteristics play important roles *across* mammalian species (Ungar, 2010). Humans are not subject to the same natural selection pressures as most other mammalian species; therefore, we believe it will be important to continue studies of intra-specific variation in oral motor control, morphology and occlusal characteristics among other mammalian species.

5. Conclusions

Chewing performance, operationally defined using a recognized standard, viz., median particle size (X_{50}), appeared to be subject-specific and related to each subject's inherent ability to reduce food on a per-chew basis. Demographic, morphological, and EMG variables played little role in performance, whereas occlusal contact area and variation in the number of chews in a trial played a significant role. In addition, variation in chewing rate played a lesser but recognizable role. Surprisingly, chewing cycle duration, EMG burst duration, and peak onset latency increased when the number of chews was restricted in the ($+N_C$,$-T_C$) task. This suggests that these EMG parameters were modulated by subjects, which consequently modified chewing performance. It was also surprising that chewing rate variability was reduced when restricting the number of chews alone, that it was difficult to reduce variability further with a metronome, and that the variability with the metronome was proportionate to that observed under uncontrolled conditions. This suggests that there is an intrinsic, individual-specific variability to chewing rate. Also, individuals increased bite force when experimental control over chewing rate variability and number of chews was added; however, bite force did not appear to play an important role in variation in performance across individuals. Overall, this study provided evidence that bite force, chewing rate, chewing performance and chewing bout duration all showed individual preferences. It will be interesting for future work to determine whether similar findings occur among other mammals, e.g., (Ross, Washington et al., 2009).

Conflict of interest

None.

Ethical approval

Ethical Approval was given by the University of Michigan medical school IRB IRB-MED; Human study HUM00087223.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.archoralbio.2018.03.007.

References

Anastassiadou, V., & Heath, M. R. (2001). The development of a simple objective test of mastication suitable for older people, using chewing gums. *Gerodontology, 18*(2), 79–86.

Barlow, S. M., Lund, J. P., Estep, M., & Kolta, A. (2010). Central pattern generators for orofacial movements and speech. In S. M. Brudzynski (Vol. Ed.), *Handbook of mammalian vocalization: 19*, (pp. 351–369). Amsterdam: Elsevier.

Bhatka, R., Throckmorton, G. S., Wintergerst, A. M., Hutchins, B., & Buschang, P. H. (2004). Bolus size and unilateral chewing cycle kinematics. *Archives of Oral Biology, 49*(7), 559–566. http://dx.doi.org/10.1016/j.archoralbio.2004.01.014.

Brezani, I., & Zelenak, F. (2010). Improving the effectivity of work with Rosin-Rammler diagram by using MATLAB GUI tool. *Acta Montanistica Slovaca, 15*(2), 152–157.

Chavez, E. M., & Ship, J. A. (2000). Sensory and motor deficits in the elderly: Impact on

oral health. *Journal of Public Health Dentistry, 60*(4), 297–303.
Cosme, D. C., Baldisserotto, S. M., Canabarro, S. D., & Shinkai, R. S. (2005). Bruxism and voluntary maximal bite force in young dentate adults. *International Journal of Prosthodontics, 18*(4), 328–332.
Diracoglu, D., Alptekin, K., Cifter, E. D., Guclu, B., Karan, A., & Aksoy, C. (2011). Relationship between maximal bite force and tooth wear in bruxist and non-bruxist individuals. *Archives of Oral Biology, 56*(12), 1569–1575. http://dx.doi.org/10.1016/j.archoralbio.2011.06.019.
Druzinsky, R. E. (1993). The time allometry of mammalian chewing movements: Chewing frequency scales with body mass in mammals. *Journal of Theoretical Biology, 160*(4), 427–440.
Dworkin, S. F., & LeResche, L. (1992). Research diagnostic criteria for temporomandibular disorders: Review, criteria, examinations and specifications, critique. *Journal of Craniomandibular Disorders, 6*(4), 301–355.
Eberhard, L., Schindler, H. J., Hellmann, D., Schmitter, M., Rammelsberg, P., & Giannakopoulos, N. N. (2012). Comparison of particle-size distributions determined by optical scanning and by sieving in the assessment of masticatory performance. *Journal of Oral Rehabilitation, 39*(5), 338–348. http://dx.doi.org/10.1111/j.1365-2842.2011.02275.x.
Engelen, L., Fontijn-Tekamp, F. A., & Van Der Bilt, A. (2005). The influence of product and oral characteristics on swallowing. *Archives of Oral Biology, 50*, 739–746.
English, J. D., Buschang, P. H., & Throckmorton, G. S. (2002). Does malocclusion affect masticatory performance? *Angle Orthodontist, 72*(1), 21–27.
Estebaranz, F., Galbany, J., Martinez, L. M., & Perez-Perez, A. (2007). 3-D interferometric microscopy applied to the study of buccal enamel microwear. In S. E. Bailey, & J. J. Hublin (Eds.). *Dental perspectives on human evolution: State of the art research in dental paleoanthropology* (pp. 391–403). Dordrecht: Springer.
Feldman, R. S., Kapur, K. K., Alman, J. E., & Chauncey, H. H. (1980). Aging and mastication: Changes in performance and in the swallowing threshold with natural dentition. *Journal of the American Geriatrics Society, 28*(3), 97–103.
Ferrario, V. F., & Sforza, C. (1996). Coordinated electromyographic activity of the human masseter and temporalis anterior muscles during mastication. *European Journal of Oral Sciences, 104*(5–6), 511–517.
Fitts, P. M. (1954). The information capacity of the human motor system in controlling the amplitude of movement. *Journal of Experimental Psychology: Human Perception & Performance, 47*(6), 381–391.
Foster, K. D., Woda, A., & Peyron, M. A. (2006). Effect of texture of plastic and elastic model foods on the parameters of mastication. *Journal of Neurophysiology, 95*, 3469–3479.
Fulks, B., Callaghan, K. X., Tewksbury, C. D., & Gerstner, G. E. (2017). Relationships between chewing rate, occlusion, cephalometric anatomy, muscle activity, and masticatory performance. *Archives of Oral Biology, 83*, 161–168.
Gerstner, G. E., & Fehrman, J. (1999). Comparison of chin and jaw movement parameters during gum chewing. *Journal of Prosthetic Dentistry, 81*, 179–185.
Gerstner, G. E., & Gerstein, J. B. (2008). Chewing rate allometry among mammals. *Journal of Mammalogy, 89*(4), 1020–1030.
Gerstner, G. E., & Goldberg, L. J. (1991). Genioglossus EMG activity during rhythmic jaw movements in the anesthetized guinea pig. *Brain Research, 562*, 79–84.
Gerstner, G. E., & Parekh, V. V. (1997). Evidence of sex-specific differences in masticatory jaw movement patterns. *Journal of Dental Research, 76*(3), 796–806.
Gerstner, G. E., Marchi, F., & Haerian, H. (1999). Relationship between anteroposterior maxillomandibular morphology and masticatory jaw movement patterns. *American Journal of Orthodontics & Dentofacial Orthopedics, 115*(3), 258–266.
Gerstner, G. E., Madhavan, S., & Braun, T. M. (2014). Relationships between masticatory rhythmicity, body mass and cephalometrically-determined aesthetic and functional variables during development in humans. *Archives of Oral Biology, 59*(7), 711–721. http://dx.doi.org/10.1016/j.archoralbio.2014.04.011.
Gintof, C., Konow, N., Ross, C. F., & Sanford, C. P. (2010). Rhythmic chewing with oral jaws in teleost fishes: A comparison with amniotes. *Journal of Experimental Biology, 213*(11), 1868–1875. http://dx.doi.org/10.1242/jeb.041012.
Gonzalez, R., Sifre, S., Benedito, J., & Nogues, V. (2002). Comparison of electromyographic pattern of sensory experts and untrained subjects during chewing of Mahon cheese. *Journal of Dairy Research, 69*(1), 151–161.
Hada, M., Tabe, T., Tsuka, H., Yamauchi, K., & Muneoka, Y. (1977). [The properties of chewing-gum as a test food for measurement of masticatory performance (author's transl)]. *Hiroshima Daigaku Shigaku Zasshi Journal of Hiroshima University Dental Society, 9*(2), 232–235.
Hayakawa, I., Watanabe, I., Hirano, S., Nagao, M., & Seki, T. (1998). A simple method for evaluating masticatory performance using a color-changeable chewing gum. *International Journal of Prosthodontics, 11*(2), 173–176.
Hayasaki, H., Saitoh, I., Throckmorton, G. S., Iwase, Y., Nakata, S., & Nakata, M. (2003). Occlusal phase of gum-chewing strokes. *Journal of Oral Rehabilitation, 30*(10), 1041–1046.
Heath, M. R. (1982). The effect of maximum biting force and bone loss upon masticatory function and dietary selection of the elderly. *International Dental Journal, 32*(4), 345–356.
Helkimo, E., Carlsson, G. E., & Helkimo, M. (1978). Chewing efficiency and state of dentition. A methodologic study. *Acta Odontologica Scandinavica, 36*(1), 33–41.
Hill, A. V. (1938). The heat of shortening and dynamics constants of muscles. *Proceedings of the Royal Society of London B: Biological Sciences, 126*(843), 136–195.
Hirano, K., Hirano, S., & Hayakawa, I. (2004). The role of oral sensorimotor function in masticatory ability. *Journal of Oral Rehabilitation, 31*(3), 199–205.
Hotta, T. H., Nunes, L. J., Quatrini, A. H., Bataglion, C., Nonaka, T., & Bezzon, O. L. (2000). Tooth wear and loss: Symptomatological and rehabilitating treatments. *Brazilian Dental Journal, 11*(2), 147–152.
Ives, J. C., & Wigglesworth, J. K. (2003). Sampling rate effects on surface EMG timing and amplitude measures. *Clinical Biomechanics, 18*(6), 543–552. http://dx.doi.org/10.1016/s0268-0033(03)00089-5.
Johansson, A., Kiliaridis, S., Haraldson, T., Omar, R., & Carlsson, G. E. (1993). Covariation of some factors associated with occlusal tooth wear in a selected high-wear sample. *Scandinavian Journal of Dental Research, 101*(6), 398–406.
Karlsson, S., Persson, M., & Carlsson, G. E. (1991). Mandibular movement and velocity in relation to state of dentition and age. *Journal of Oral Rehabilitation, 18*(1), 1–8.
Kiliaridis, S., Johansson, A., Haraldson, T., Omar, R., & Carlsson, G. E. (1995). Craniofacial morphology, occlusal traits, and bite force in persons with advanced occlusal tooth wear. *American Journal of Orthodontics & Dentofacial Orthopedics, 107*(3), 286–292.
King, S. J., Arrigo-Nelson, S. J., Pochron, S. T., Semprebon, G. M., Godfrey, L. R., Wright, P. C., & Jernvall, J. (2005). Dental senescence in a long-lived primate links infant survival to rainfall. *Proceedings of the National Academy of Sciences of the United States of America, 102*(46), 16579–16583. http://dx.doi.org/10.1073/pnas.0508377102.
Kohyama, K., Mioche, L., & Bourdiol, P. (2003). Influence of age and dental status on chewing behaviour studied by EMG recordings during consumption of various food samples. *Gerodontology, 20*(1), 15–23.
Kojola, I., Helle, T., Huhta, E., & Niva, A. (1998). Foraging conditions: Tooth wear and herbivore body reserves: A study of female reindeer. *Oecologia, 117*, 26–30.
Laird, M. F., Vogel, E. R., & Pontzer, H. (2016). Chewing efficiency and occlusal functional morphology in modern humans. *Journal of Human Evolution, 93*, 1–11.
Laurell, L., & Lundgren, D. (1985). Chewing ability in patients restored with cross-arch fixed partial dentures. *Journal of Prosthetic Dentistry, 54*(5), 720–725.
Lepley, C., Throckmorton, G. S., Parker, S., & Buschang, P. H. (2010). Masticatory performance and chewing cycle kinematics-are they related? *Angle Orthodontist, 80*(2), 295–301.
Liedberg, B., & Owall, B. (1995). Oral bolus kneading and shaping measured with chewing gum. *Dysphagia, 10*(2), 101–106.
Lucas, P. W., & Omar, R. (2012). Damaged! A new overview of dental wear. *Archives of Oral Biology, 57*(3), 211–213. http://dx.doi.org/10.1016/j.archoralbio.2011.11.001.
Luke, D. A., & Lucas, P. W. (1985). Chewing efficiency in relation to occlusal and other variations in the natural human dentition. *British Dental Journal, 159*(12), 401–403.
Lund, J. P., & Kolta, A. (2006). Generation of the central masticatory pattern and its modification by sensory feedback. *Dysphagia, 21*(3), 167–174. http://dx.doi.org/10.1007/s00455-006-9027-6.
Lund, J. P. (1991). Mastication and its control by the brain stem. *Critical Reviews in Oral Biology & Medicine, 2*(1), 33–64.
Magalhaes, I. B., Pereira, L. J., Marques, L. S., & Gameiro, G. H. (2010). The influence of malocclusion on masticatory performance. A systematic review. *Angle Orthodontist, 80*(5), 981–987. http://dx.doi.org/10.2319/011910-33.1.
Mahoney, P. (2006). Microwear and morphology: Functional relationships between human dental microwear and the mandible. *Journal of Human Evolution, 50*(4), 452–459. http://dx.doi.org/10.1016/j.jhevol.2005.11.003.
Marquezin, M. C., Kobayashi, F. Y., Montes, A. B., Gaviao, M. B., & Castelo, P. M. (2013). Assessment of masticatory performance, bite force, orthodontic treatment need and orofacial dysfunction in children and adolescents. *Archives of Oral Biology, 58*(3), 286–292. http://dx.doi.org/10.1016/j.archoralbio.2012.06.018.
Miura, H., Miura, K., Mizugai, H., Arai, Y., Umenai, T., & Isogai, E. (2000). Chewing ability and quality of life among the elderly residing in a rural community in Japan. *Journal of Oral Rehabilitation, 27*(8), 731–734.
N'Gom P, I., & Woda, A. (2002). Influence of impaired mastication on nutrition. *Journal of Prosthetic Dentistry, 87*(6), 667–673.
Nakamura, Y., & Katakura, N. (1995). Generation of masticatory rhythm in the brainstem. *Neuroscience Research, 23*, 1–19.
Olthoff, L. W., van der Bilt, A., Bosman, F., & Kleizen, H. H. (1984). Distribution of particle sizes in food comminuted by human mastication. *Archives of Oral Biology, 29*(11), 899–903.
Owens, S., Buschang, P. H., Throckmorton, G. S., Palmer, L., & English, J. (2002). Masticatory performance and areas of occlusal contact and near contact in subjects with normal occlusion and malocclusion. *American Journal of Orthodontics and Dentofacial Orthopedics, 121*(6), 602–609. http://dx.doi.org/10.1067/mod.2002.122829.
Palmer, J. B., Rudin, N. J., Lara, G., & Crompton, A. W. (1992). Coordination of mastication and swallowing. *Dysphagia, 7*(4), 187–200.
Park, I. H., McCall, W. D., Jr., & Chung, J. W. (2012). Electromyographic power spectrum of jaw muscles during clenching in unilateral temporomandibular joint osteoarthritis patients. *Journal of Oral Rehabilitation, 39*(9), 659–667. http://dx.doi.org/10.1111/j.1365-2842.2012.02322.x.
Pereira, L. J., Duarte Gaviao, M. B., & Van Der Bilt, A. (2006). Influence of oral characteristics and food products on masticatory function. *Acta Odontologica Scandinavica, 64*(4), 193–201. http://dx.doi.org/10.1080/00016350600703459.
Peyron, M. A., Lassauzay, C., & Woda, A. (2002). Effects of increased hardness on jaw movement and muscle activity during chewing of visco-elastic model foods. *Experimental Brain Research, 142*(1), 41–51.
Peyron, M. A., Mishellany, A., & Woda, A. (2004). Particle size distribution of food boluses after mastication of six natural foods. *Journal of Dental Research, 83*(7), 578–582.
Peyron, M. A., Woda, A., Bourdiol, P., & Hennequin, M. (2017). Age-related changes in mastication. *Journal of Oral Rehabilitation, 44*, 299–312.
Prinz, J. F. (2004). Abrasives in foods and their effect on intra-oral processing: A two-colour chewing gum study. *Journal of Oral Rehabilitation, 31*, 968–971.
Reed, D. A., & Ross, C. F. (2010). The influence of food material properties on jaw kinematics in the primate, Cebus. *Archives of Oral Biology, 55*, 946–962.
Rosin, P., & Rammler, E. (1933). Gesetzmassigkeiten in der Kornzusammensetzung des Zementes. *Zement, 31*, 427–433.

Ross, C. F., Baden, A. L., Georgi, J., Herrel, A., Metzger, K. A., Reed, D. A., ... Wolff, M. S. (2010). Chewing variation in lepidosaurs and primates. *Journal of Experimental Biology, 213*, 572–584.

Ross, C. F., Dharia, R., Herring, S. W., Hylander, W. L., Liu, Z.-J., Rafferty, K. L., ... Williams, S. H. (2007). Modulation of mandibular loading and bite force in mammals during mastication. *Journal of Experimental Biology, 210*, 1046–1063.

Ross, C. F., Eckhardt, A., Herrel, A., Hylander, W. L., Metzger, K. A., Schaerlaeken, V., ... Williams, S. H. (2007). Modulation of intra-oral processing in mammals and lepidosaurs. *Integrative and Comparative Biology, 47*, 118–136.

Ross, C. F., Reed, D. A., Washington, R. L., Eckhardt, A., Anapol, F., & Shahnoor, N. (2009). Scaling of chew cycle duration in primates. *American Journal of Physical Anthropology, 138*, 30–44.

Ross, C. F., Washington, R. L., Eckhardt, A., Reed, D. A., Vogel, E. R., Dominy, N. J., & Machanda, Z. P. (2009). Ecological consequences of scaling of chewing cycle duration and daily feeding time in Primates. *Journal of Human Evolution, 56*, 570–585.

Sanchez-Ayala, A., Farias-Neto, A., Campanha, N. H., & Garcia, R. C. M. R. (2013). Relationship between chewing rate and masticatory performance. *Journal of Craniomandibular Practice, 31*(2), 118–122.

Schwartz, G., Enomoto, S., Valiquette, C., & Lund, J. P. (1989). Mastication in the rabbit: A description of movement and muscle activity. *Journal of Neurophysiology, 62*(1), 273–287.

Smith, K. K. (1992). The evolution of the mammalian pharynx. *Zoological Journal of the Linnean Society, 104*, 313–349.

Solounias, N., Fortelius, M., & Freeman, P. (1994). Molar wear rates in ruminants – A new approach. *Annales Zoologici Fennici, 31*(2), 219–227.

Stepp, C. E. (2012). Surface electromyography for speech and swallowing systems: Measurement, analysis, and interpretation. *Journal of Speech, Language, and Hearing Research, 55*, 1232–1246. http://dx.doi.org/10.1044/1092-4388(2011/11-0214.

Toro, A., Buschang, P. H., Throckmorton, G. S., & Roldan, S. (2005). Masticatory performance in children and adolescents with Class I and II malocclusions. *European Journal of Orthodontics, 28*, 112–119.

Tyler, N. J. C. (1986). The relationship between the fat content of Svalbard reindeer in autumn and their death from starvation in winter. *Rangifer, 1*, 311–314 Special Issue.

Ungar, P. S. (2010). *Mammal teeth: Origin, evolution and diversity.* Baltimore: The Johns Hopkins University Press.

Veiberg, V., Mysterud, A., Gaillard, J. M., Delorme, D., Van Laere, G., & Klein, F. (2007). Bigger teeth for longer life? Longevity and molar height in two roe deer populations. *Biology Letters, 3*(3), 268–270. http://dx.doi.org/10.1098/rsbl.2006.0610.

Wetselaar, P., Vermaire, J. H., Visscher, C. M., Lobbezoo, F., & Schuller, A. A. (2016). The prevalence of tooth wear in the Dutch adult population. *Caries Research, 50*(6), 543–550. http://dx.doi.org/10.1159/000447020.

Wilding, R. J. (1993). The association between chewing efficiency and occlusal contact area in man. *Archives of Oral Biology, 38*(7), 589–596.

Wright, P., King, S. J., Baden, A., & Jernvall, J. (2008). Aging in wild female lemurs: Sustained fertility with increased infant mortality. *Primate Reproductive Aging: Cross-Taxon Perspectives, 36*, 17–28.

Yamashita, S., Hatch, J. P., & Rugh, J. D. (1999). Does chewing performance depend upon a specific masticatory pattern? *Journal of Oral Rehabilitation, 26*(7), 547–553.

van den Braber, W., van der Glas, H. W., van der Bilt, A., & Bosman, F. (2001). Chewing efficiency of pre-orthognathic surgery patients: Selection and breakage of food particles. *European Journal of Oral Sciences, 109*(5), 306–311.

van der Bilt, A., Olthoff, L. W., Bosman, F., & Oosterhaven, S. P. (1993). The effect of missing postcanine teeth on chewing performance in man. *Archives of Oral Biology, 38*(5), 423–429.