Mechanical and Thermal Food Processing Effects on Mastication and Cranio-Dental Morphology

Mechanical and thermal food processing effects on mastication and cranio-dental morphology

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ABSTRACT

Chimpanzees spend ~40% of their day chewing fruits, seeds, and tough leaves and pith, while in contrast modern humans spend significantly less time eating (5%), and the foods that they consume are extremely soft and processed. How have these differences, especially the advent and increasing use of foods processing techniques, influenced masticatory effort and ultimately the morphology of the jaws and teeth? This dissertation addresses this question by measuring the effects that early hominin food processing methods (slicing, pounding, and roasting) have on food material properties, masticatory performance and functional integration of the teeth and jaws.

Using standard testing techniques, the material properties of plant tubers and meat were quantified. Processing had contrasting effects on the properties of these foods, and were correlated with masticatory performance changes measured in human experiments. Mechanical processing techniques decreased tuber toughness, leading to lower chew force (CF). Roasting further decreased tuber toughness and other material properties, which led to lower comminution efficiency (CE) and CF. In direct contrast to tubers, mechanical processing techniques did not alter meat toughness, yet did increase CF and CE. Roasting the meat also increased CF and CE, likely because of higher toughness and stiffness, coupled with less elastic energy loss.

The generation of lower masticatory forces resulting from processing have undoubtedly affected cranio-dental morphology. In particular, it is hypothesized that forces functionally integrate the masticatory system, and reduced forces, especially in modern human populations, lead to malocclusions (dis-integration). An animal experiment was performed to test this hypothesis, and the results indicate

that masticatory effort (eating hard or soft foods) coordinates jaw and dental growth. Further testing the hypothesis, the effects of morphology on masticatory function were studied by coupling subject masticatory performance with occlusal scores. Multiple regressions of occlusion and tooth size explained a high proportion of masticatory performance variance (significantly more than tooth size alone), suggesting that occlusal integration does indeed affect masticatory function. Taken together, the results of this dissertation document the significant reductions in hominin masticatory forces and changes in cranio-dental growth and integration that may have resulted from the use of food processing techniques.

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CHAPTER 1: INTRODUCTION

Humans are the only animal that relies heavily on extra-oral food processing. We not only cook much of our food, but also mechanically or chemically process almost everything we put in our mouths. In fact, we may be so biologically dependent on processed foods that it is not possible for humans to survive without substantial food processing. How does food processing affect how humans chew, the forces that are generated, and the growth of the jaws? How, also, has our reliance on food processing affected the coevolution of hominin faces and teeth over the last few million years? There has been a marked reduction of postcanine tooth size within the genus *Homo* (Brace, 1967; Wolpoff, 1973; McHenry, 1994) and overall buttressing and robustness of the face has also decreased (for review, see (Chamberlain and Wood, 1985; Lahr and Wright, 1996; Wood and Collard, 1999; Lieberman, 2011)). While the selective forces that drove these reductions in *Homo* are unclear, they must have been made possible by changes in the diet such as the consumption of softer foods that require less force per chew or fewer chews to comminute, along with higher quality foods that pack more calories per unit volume and necessitate less consumption overall. A shift to higher quality diet is further hypothesized to underlie relatively larger brains and smaller intestines, absolutely larger bodies, shorter inter-birth intervals, larger home ranges and other key variables that are affected by energy availability (e.g., (Aiello and Wheeler, 1995; Wrangham et al., 1999; Kaplan et al., 2000; Aiello and Key, 2002; Aiello and Wells, 2002; Wrangham, 2009)).

Reduced size of the masticatory apparatus coupled with evidence of increased energetic demands has focused much attention on the dietary shifts that may have occurred during the early evolution of the genus *Homo*. Two of the most discussed possibilities include the addition of more meat to the diet (e.g., (Hill, 1982; Shipman, 1986; Milton, 1999; Stanford and Bunn, 2001; Bunn, 2007)) and the adoption of food processing techniques (e.g., (Wrangham et al., 1999; Lucas, 2004; Wrangham, 2009; Lieberman, 2011)). Dental microwear and carbon isotopic analyses indicate that early *Homo*, and especially *H. erectus*, had broad dietary niches and consumed a mixture of C_3 and C_4 foods that, on

average, were not particularly hard or tough (Lee-Thorp et al., 2000; Ungar et al., 2006b; van der Merwe et al., 2008; Pontzer et al., 2011; Ungar and Sponheimer, 2011; Ungar et al., 2012). There is also abundant evidence that *Homo* consumed more animal products than earlier hominins. Recently, Balter et al. (2012) analyzed Sr/Ca and Ba/Ca ratios in the molar enamel of South African early *Homo* and australopithecines. They found signals in *Homo* similar to those of carnivores, providing strong evidence that early *Homo* was consuming meat products in much larger quantities than australopithecines from the same locale. This finding is not surprising. Abundant archaeological evidence, such as stone tools and bone cut marks, indicate increased meat consumption by Oldowan hominins (Bunn, 1981; Bunn and Kroll, 1986; Bunn, 1994; Dominguez-Rodrigo et al., 2002; Plummer, 2004; Dominguez-Rodrigo and Barba, 2006; Bunn, 2007). The potential benefits from meat are numerous: it is an extremely valuable food source that is calorically dense, fully digestible, and provides important sources of protein and fat. From a biomechanical perspective, however, raw meat consumption is a challenge and is thought to require considerable effort for hominins to comminute (break down) through mastication. Muscle tissue is highly extensible and tends to blunt fractures, keeping them from propagating. In order to efficiently fracture meat in the space-limited environment of two occluding teeth, shearing crests are needed. Apes in general, and hominins in particular, have low-crested bunodont molars that are unable to effectively chew meat. According to some accounts, chimpanzees can spend 4-11.5 hours chewing animal carcasses weighing approximately 4 kg (Goodall, 1986; Wrangham and Conklin-Brittain, 2003). These observations suggest that increased meat consumption by hominins may have required substantially more chewing effort, which is not consistent with the reduced cranio-dental size and robusticity of *Homo* species.

Another dietary shift that may have evolved in early *Homo*, one that is not mutually exclusive to increased meat eating, is the adoption of food processing techniques. Without exception, all modern human populations process much of their food before consumption (Wrangham and Conklin-Brittain,

2003; Wrangham, 2007). Today we puree, fry, boil, bake, and steam, and even modern day 'raw foodists' who eschew thermal heating of food, expend much effort dehydrating, grinding, blending, juicing and soaking (Baird and Rodwell, 2005). Of all methods, cooking is the most discussed type of food processing because it may have played an especially important role in the evolution of the human genus (e.g. (Wrangham et al., 1999; Wrangham and Conklin-Brittain, 2003; Carmody and Wrangham, 2009; Wrangham, 2009; Carmody et al., 2011)). Some researchers have suggested that the origin of cooking may date as far back as the evolution of *H. erectus*. For example, Wrangham et al. (1999) hypothesized that since cooking softens foods and increases net nutrient availability (by increasing digestibility and/or decreasing cost of digestion), it may have made possible the increased brain and body size and smaller guts, teeth and less robust faces of *H. erectus*. Additionally, Lucas (2004) has used fracture scaling mechanics to explain the pattern of differential size decreases of anterior and posterior teeth in early *H. erectus* as a result of lowered food toughness caused by roasting foods such as tubers. When cooking began, however, is controversial. One problem with the 'cooking hypothesis', is a lack of evidence for controlled fire, let alone cooking use, as far back as early *H. erectus*. Until recently, the oldest clear evidence of fire in the archeological record was from Gesher Bonet Ya'aqov at 790 kya (Goren-Inbar et al., 2004). New evidence of fire (burned bone and plant ashes) in Wonderwerk Cave, South Africa has pushed this date to 1 mya (Berna et al., 2012). Even so, hearths and other features indicative of cooking are not common until the middle Paleolithic (see James (1989) for review), leading many researchers to believe that frequent, habitual cooking is a relatively recent behavior (e.g., (Brace, 1995; Ragir, 2000; Bunn, 2007)). The evidence of fire use at 1 mya in South Africa has revived discussion on this topic, however, and may lead to renewed effort to test for signs of cooking in the Early Stone Age (Roberts and Bird, 2012).

Debates over cooking have overshadowed other forms of food processing, such as mechanical processing, which almost certainly pre-date the use of fire and were almost definitely employed by early

Homo. Chimpanzees sometimes use rudimentary tools to pound open hard nuts (Goodall, 1986; Boesch and Boesch-Achermann, 2000) and other animals, such as otters, use stones to break open hard mollusk shells (Hall and Schaller, 1964). It is therefore reasonable to expect that the last common ancestor of chimpanzees and hominins also sometimes practiced this form of rudimentary processing. By the time early *Homo* evolved, mechanical alteration of food likely became much more complex. The oldest stone tools date to approximately 2.6 mya (Semaw et al., 1997), and tool cut marks are argued to be present on animal bones dating to as early as 3.4 mya (McPherron et al., 2010). Analyses of tools and bone cut marks at early Oldowan sites indicate that hominins were using these stones extensively to cut meat and possibly plant material (Keeley and Toth, 1981; Semaw et al., 2003; Dominguez-Rodrigo et al., 2005; Bunn, 2007; Pobiner et al., 2008). Sharp edges on hand axes could have been used to slice meat into smaller, more easily ingested particles that required less chewing. In addition, many Lower Paleolithic tools such as spheroids, hammerstones and handaxes could have been used to pound and possibly grind food. These mechanical processing methods might have significantly reduced masticatory effort by reducing ingested particle size and tenderizing the food.

A major difficulty with testing various dietary hypotheses, however, is that it is not known if and by how much the inclusion of more meat in the diet and/or the adoption of food processing techniques could have permitted smaller postcanine dentition and other related craniofacial changes evident in the genus *Homo*. Chewing produces high, repetitive forces that must be resisted by the teeth and skull. Chimpanzees subsist largely on fruits, seeds, and tough leaves and pith, and researchers observe that on average they spend approximately 40% of their day chewing (Organ et al., 2011). In contrast, modern humans consume a soft, processed diet that requires relatively little effort to masticate, and spend only a small fraction (~5%) of their day eating (Organ et al., 2011). This drastic difference in masticatory effort has a number of cranio-dental implications. Larger teeth provide more occlusal area to distribute high chewing forces, and teeth with relatively thicker enamel may be better able to withstand fractures

caused by high bite forces and/or attrition from plant phytoliths, or more likely, exogenous grit (Lucas et al., 2008; Constantino et al., 2009; Rabenold and Pearson, 2011; Lucas et al., 2013). Additionally, chewing forces generate bone stresses (force per area), which strain and deform the bone. Too much strain causes potentially deleterious micro-fractures in the bone, and the skull adapts to high chewing forces by adding bone mass in the plane of deformation to reduce overall stress. Chewing forces produce a complex pattern of wishbone, twisting, bending and shearing strain in the face and jaws (Hylander, 1984; Hylander et al., 1991; Hylander and Johnson, 1994; Ross and Hylander, 1996; Hylander and Johnson, 1997; Daegling and Hylander, 1998; Ross, 2001; Lieberman et al., 2004), and larger, more robust faces and jaws, such as those seen in chimpanzees and australopiths, are better able to withstand those strains. The correlation between morphology and chewing effort is most evident when one considers the anatomy of the robust australopiths (*A. boisei* and *A. robustus*). With the largest postcanine teeth, jaw bones, and chewing musculature of all hominins, as well as wide, flat faces capable of withstanding high chewing strains, it is clear that the robust australopiths consumed, at least on occasion, foods that required a substantial amount of masticatory effort to comminute (e.g., (Rak, 1983; Chamberlain and Wood, 1985; Demes and Creel, 1988; McCollum, 1994; Wood and Aiello, 1998; Lieberman, 2011)).

While masticatory forces can shape the teeth and face over evolutionary time, morphological changes also occur within an animal's life span. A large number of feeding experiments have demonstrated that animals fed soft foods tend to develop smaller mandibular corpora, dental arches and palates, and shorter mandibular rami than animals subsisting on hard foods (e.g. (Watt and Williams, 1951; Beecher and Corruccini, 1981; Corruccini and Beecher, 1982; Beecher et al., 1983; Corruccini and Beecher, 1984; Ciochon et al., 1997; Tokimasa et al., 2000; Maki et al., 2002; Lieberman et al., 2004)). Interestingly, in addition to general facial size decreases, animals with low masticatory loading also appear to be significantly more prone to developing malocclusions (Corruccini and Beecher,

1982; Beecher et al., 1983; Larsson et al., 2005) and exhibit more intra-group morphological variability (Corruccini and Beecher, 1984). Why is this the case? One possibility is that a certain amount of chewing force is necessary to properly integrate the growth of the upper and lower jaws. Most experiments on dietary loading feed animals an extremely soft diet consisting of liquefied or powdered food that effectively removes most masticatory loading. This experimental condition may be particularly relevant to modern humans. Occlusal health in modern western societies is markedly low, and close to 50% of the population in the U.S. is afflicted by moderate to major dental problems such as tooth displacements, dental rotations, overjets, and openbites (Kelly and Harvey, 1977). These occlusal variations underline what happens when normal integrative processes fail and are not present at such levels in wild primates groups and non-industrial human populations (Mills, 1963; Corruccini, 1984; Corruccini, 1999; Evensen and Øgaard, 2007).

One of the most pervasive hypotheses in the anthropological literature to explain the recent malocclusion epidemic involves the idea of 'disuse' (see Corruccini (1999) for review). According to this hypothesis, chewing soft, highly processed food does not produce the stresses necessary to stimulate proper growth and alignment of the jaws and dentition. This idea is supported by relatively low heritabilities for cranio-dental features and measures of dental misalignments (Boraas et al., 1988; Cassidy et al., 1998; Hughes et al., 2000; Hughes et al., 2001; Townsend et al., 2003; Eguchi et al., 2004), as well as comparative malocclusion studies of aboriginal/rural vs. modernized/urban populations (e.g., (McCann et al., 1966; Niswander, 1967; Lavelle, 1968; Corruccini and Whitley, 1981; Corruccini, 1999; Evensen and Øgaard, 2007)).

Although the effects of a modern, western diet on cranio-dental morphology may represent a unique case whereby extreme food processing has resulted in reduced integration, it nonetheless highlights the substantial changes in cranio-dental function and morphology likely associated with simple food processing. It is reasonable to hypothesize that humans evolved to process food to a

certain, but limited extent. This has resulted in the development of less robust faces and smaller teeth in *Homo*. Modern food processing methods may have altered these conditions to pathologically bad levels, resulting in malocclusions. In order to begin to understand the effects of food processing on morphology, however, it is first necessary to understand how mastication (e.g. the force generated) is altered by food processing. Therefore, the major goal of this dissertation is to provide experimental data on the extent to which simple mechanical processing and cooking methods affect the material properties of food that are relevant to mastication, and the resulting changes on masticatory performance.

Objectives

The first two data chapters in this dissertation experimentally quantify the material property (Chapter 2) and masticatory performance (Chapter 3) changes that result from simple cooking and mechanical processing methods available to hominins, dry roasting, slicing and pounding. Although many potential food types can be studied, this dissertation focuses on the effect of processing tubers and meat. These foods are thought to have been important components of hominin diets (e.g., (Hatley and Kappelman, 1980; Milton, 1999; Laden and Wrangham, 2005; Ungar et al., 2006a; Bunn, 2007)) and are extremely dissimilar from a materials standpoint, which might cause a differential response to processing: raw vegetables cells are under significant internal turgor pressure, while meat is made up of elastic muscle fibers and connective tissue.

The data from Chapters 2 and 3 are combined to address three specific objectives. First, I analyze how mechanical processing (slicing and pounding) versus cooking affects material properties and masticatory performance. The general prediction tested is that slicing, pounding and roasting improve chewing performance in humans by facilitating comminution (intra-oral food fragmentation) and decreasing masticatory forces.

Second, the relationship between the material properties of raw and processed foods and the masticatory force required to consume them is assessed. This is particularly important because masticatory performance experiments can be time and cost-intensive, precluding the testing of a wide range of food and processing types. In contrast, material property data are more readily measured. Agrawal et al. (1997 and 1998) showed a strong correlation between a food's material properties and both masticatory muscle activity and food fragmentation after a single bite. It is therefore reasonable to predict that the material property changes associated with processing will also be good predictors of differences in masticatory force. If this is the case, then the large number of foods and processing techniques that can be tested will further enlighten hominin dietary hypotheses.

Finally, the masticatory performance data for meat and tubers are used to model hominin masticatory force changes resulting from the addition of more meat in the diet and/or the adoption of food processing techniques. Is the addition of more meat in the diet of *Homo* sufficient to explain their dental and masticatory size reductions? If not, then the early adoption and regular use of food processing is supported. And if this is the case, what are the relative effects of mechanical processing versus thermal (cooking) techniques on masticatory effort?

The last data chapter in this dissertation, Chapter 4, focuses on the integration of the teeth and jaws. The results from two studies are presented. First, the masticatory performance data collected in Chapter 3 is coupled with subject dental morphology to examine the link between occlusal integration and masticatory performance. Although the data collected in this dissertation cannot test how or if the masticatory system remains integrated in light of changing forces, it does provide an opportunity to test experimentally whether the strength of morphological integration (based on objective scores of the subjects' occlusion) does indeed affect function, and if this relationship changes depending on whether the food is processed or raw. This functional analysis is then followed by a preliminary animal experiment that tests if masticatory forces coordinate bone and dental morphology.

The data chapters are followed by a brief culminating chapter (Chapter 5) that ties together the results of the studies described within this dissertation. As part of this chapter, conclusions and areas of future research are outlined and discussed.

CHAPTER 2. FOOD MATERIAL PROPERTIES

INTRODUCTION

The evolution of the genus *Homo* is marked by reduction in the size of the masticatory apparatus. Overall robustness and buttressing of the face decreased, and postcanine crown area became approximately 35% smaller from gracile australopiths to *H. sapiens* (Brace, 1967; Wolpoff, 1973; Chamberlain and Wood, 1985; McHenry, 1994; Lahr and Wright, 1996; Wood and Collard, 1999; Lieberman, 2011). These morphological changes signal reduced masticatory effort within the genus, and must have been made possible by a change in diet to softer foods and/or higher quality, energetically dense foods that require fewer chews per calorie consumed. A higher quality diet is thought to further explain the larger bodies, relatively larger brains, and smaller intestines of *Homo* (especially later taxa) compared to australopithecines (e.g., (Aiello and Wheeler, 1995; Wrangham et al., 1999; Kaplan et al., 2000; Aiello and Key, 2002; Aiello and Wells, 2002)).

Lowered masticatory and digestive effort (suggested by reduced intestine size), coupled with the increased energetic demands of larger bodies and brains has focused much attention on dietary shifts that might have occurred early in the genus *Homo*. One much discussed possibility is the addition of more meat to the diet (e.g., (Milton, 1999; Bramble and Lieberman, 2004; Bunn, 2007)), which is supported by archaeological evidence such as bone cut marks and stone tool remains (Bunn, 1981; Bunn and Kroll, 1986; Bunn, 1994; Dominguez-Rodrigo et al., 2002; Plummer, 2004; Dominguez-Rodrigo and Barba, 2006; Bunn, 2007). Meat is a high quality food source; it is calorically dense, fully digestible, and provides important sources of protein and fat. From a masticatory perspective, however, consumption of raw meat may be a challenge. Muscle tissue comprises elastic contractile fibers hierarchically bound by connective tissue. Under compressive environments like the space between occluding teeth, meat fractures are blunted and do not propagate. The low-crested bunodont molars of apes and hominins appear to be especially poor at fracturing meat, and according to some accounts it takes chimpanzees 4-11.5 hours to chew small (~4 kg) animal carcasses (Goodall, 1986; Wrangham and Conklin-Brittain,

2003). These observations suggest that increased raw meat consumption by hominins may have required substantially more chewing effort, which is not consistent with the relatively smaller, less robust masticatory apparatus of *Homo* species.

Another dietary shift that likely evolved in early *Homo* is increased reliance on food processing techniques. All human populations process much of their food before consumption (Wrangham and Conklin-Brittain, 2003; Wrangham, 2007). We fry, boil, bake and steam, and even modern day 'raw foodists' who eschew thermal heating of food, expend much effort dehydrating, pureeing, blending, juicing and soaking (Baird and Rodwell, 2005). Although many food processing techniques likely evolved recently within *H. sapiens*, some of them, such as simple mechanical processing and cooking, may have been particularly important for early members of the genus *Homo*.

Tool use and very rudimentary forms of mechanical food processing are not unique to humans. For example, chimpanzees use stones to pound open hard nuts (Goodall, 1986; Boesch and Boesch-Achermann, 2000) and other animals, such as otters, use stones to break open hard mollusk shells (Hall and Schaller, 1964). It is therefore reasonable to expect that the last common ancestor of chimpanzees and hominins also practiced some form of rudimentary processing. By the time early *Homo* evolved, however, mechanical alteration of food likely became much more complex. Stone tools date to approximately 2.6 mya in the archeological record (Semaw et al., 1997), and may be even older (McPherron et al., 2010). Analyses at early Oldowan sites indicate that hominins were using these stones extensively on meat and possibly plant material (Keeley and Toth, 1981; Semaw et al., 2003; Dominguez-Rodrigo et al., 2005; Bunn, 2007; Pobiner et al., 2008). Sharp edges on hand axes could have been used to slice meat and tubers into smaller, more easily ingested particles, while Lower Paleolithic tools including spheroids, hammerstones and handaxes could have been used to pound or grind food. These different kinds of mechanical processing might have significantly reduced masticatory effort by reducing ingested particle size and tenderizing the food.

While it is clear that early *Homo* had access to mechanical food processing techniques, the timing of cooking is much more controversial. Wrangham et al. (1999) hypothesized that cooking softens foods and increases net nutrient availability (by increasing digestibility and/or decreasing cost of digestion) and this may have made possible the larger brains and body size, yet smaller guts, teeth and less robust faces of *H. erectus*. Furthering this argument, recent research has demonstrated that cooking significantly reduces cost of digestion and increases net energy gain in pythons and mice (Boback et al., 2007; Carmody et al., 2011). A major problem with ascribing cooking to *H. erectus*, however, is a lack of evidence for controlled fire, let alone cooking use, that early in human evolution. While the oldest clear evidence of fire in the archeological record is from Gesher Bonet Ya'aqov at 790 kya (Goren-Inbar et al., 2004), and recent evidence of burned bone and plants in South Africa may push this date to 1 mya (Berna et al., 2012), hearths and other features indicative of cooking do not become common until the middle Paleolithic (see James (1989) for review). This absence leads many researchers to believe that frequent cooking is a relatively recent behavior (e.g., (Brace, 1995; Ragir, 2000; Bunn, 2007)).

For all of the discussion on diet and food processing, we do not know if and by how much early processing techniques could have permitted smaller postcanine dentition and other craniofacial changes evident in the genus *Homo*. Therefore, the major goal of this study is to provide experimental data on the material property changes associated with Lower Paleolithic types of food processing. Material properties describe how a food deforms and when and how it will fracture. These intrinsic properties govern the probability of food fracture in the oral cavity and the forces necessary to create these fractures.

A number of studies have tested the effects of food characteristics (particularly hardness and other material properties) on masticatory kinematics and performance (e.g. (Hiiemae et al., 1996; Agrawal et al., 1997; Agrawal et al., 1998; Mioche et al., 1999; Agrawal et al., 2000; Okiyama et al., 2003; Kohyama et al., 2004a; Gambareli et al., 2007; Kohyama et al., 2007; Reed and Ross, 2010; Iriarte-Diaz et

al., 2011)), however most use artificial test foods (i.e. jelly gummies) or compare foods with large property differences (i.e. bananas versus almonds), and with the exception of Kohyama et al. (2007), which quantified masticatory muscle EMG changes associated with mincing foods, none of these studies have tested the effects that food processing has on masticatory performance. Additionally, although there is much research devoted to testing food material properties, most studies examine the properties of raw foods or highly processed foods such as biscuits and cheeses, and/or they measure the effects of harvest age, storage, chemical tenderizing, freeze drying, etc. on properties that relate to the taste/attractiveness of commercial foods (e.g. (Agrawal et al., 1997; Lillford, 2001; Christensen et al., 2003; Beleia et al., 2004b; Goh et al., 2005; Sui et al., 2006; Dominy et al., 2008; Vogel et al., 2008; Chang et al., 2010; Duan et al., 2010; Dilek et al., 2011)).

In comparison, relatively little research has focused on the material property changes in naturally-occurring foods caused by simple cooking or mechanical tenderization. Dominy et al. (2008), measured the material property changes induced by roasting five species of tubers eaten by Hadza hunter-gatherers, and the effects of boiling, steaming and/or microwave cooking is well documented for a number of fruits and vegetables (e.g., (Greve et al., 1994a; Greve et al., 1994b; Ng and Waldron, 1997; Thiel and Donald, 2000; Alvarez and Canet, 2001; Dan et al., 2003; Beleia et al., 2004a; Beleia et al., 2004b; Lucas, 2004)). There is extensive literature in the meat sciences on a diversity of meat types and processing methods, including forms of mechanical (blade) tenderization. However, most studies measure either Warner-Bratzler 'shear forces' (an empirical test measuring maximum fracture force with a notched blade) or perform a texture profile analysis (compressive tests that assess "hardness", "cohesiveness", "springiness" and "chewiness") as proxies for consumer sensory descriptions of a food (e.g., (Loucks et al., 1984; Mittal et al., 1992; Combes et al., 2003; King et al., 2003; de Huidobro et al., 2005; Pietrasik and Shand, 2005; Dixon et al., 2012). These are not true material properties (see below), and therefore have limited utility for modeling food fracture within the oral cavity. There have been a

few studies, however, of the specific material properties of cooked meat. The most notable is Purslow (1985), which characterized the fracture properties of beef cooked in a water bath. This was followed by a series of papers that analyzed muscle fiber and surrounding connective tissue responses to water-bath cooking (Lewis and Purslow, 1989; Mutungi et al., 1995; Willems and Purslow, 1997; Christensen et al., 2000; Christensen et al., 2004). Unfortunately, all of these studies cooked the meat by boiling it in a bag, and while this setup allows for precise control of cooking conditions, it likely results in substantially less water loss than other cooking methods.

This study builds on the existing food material property literature by measuring the food material property changes that result from using two processing techniques that were available to early hominins: simple mechanical tenderization (i.e. pounding) and dry roasting. Pounding requires little effort, time or manipulative ability and could be easily performed by any hominin with an Oldowan toolkit. Dry roasting requires no other technology aside from a fire. Experiments focus on tubers and meat because they were likely two important components of hominin diets (e.g., (Hatley and Kappelman, 1980; Milton, 1999; Laden and Wrangham, 2005; Ungar et al., 2006a; Bunn, 2007)). In addition, these are extremely different foods from a materials standpoint. Raw vegetables such as tubers comprise a latticework of cells under internal turgor pressure and can be modeled as fluid-filled foams (Gibson and Ashby, 1997). In contrast, raw meat is composed of elastic contractile fibers hierarchically bound together with connective tissue. It is therefore reasonable to expect that they will respond differently to processing.

Food Fracture

Before discussing specific hypotheses, it is helpful to first describe food fracture in the oral cavity and define the five parameters that are measured in this study: fracture stress, fracture strain, stiffness, toughness, and energy loss. Figure 2.1 shows a food item modeled as a rigid beam between

upper and lower teeth (Agrawal et al., 1997). As the teeth come into occlusion, a stress (force per area) is applied to the food, which deforms as it absorbs strain energy. As stress increases, deformation becomes permanent (yield stress) and eventually the food fractures (fracture stress). Cracks can be initiated by indentation at the cusp tips or by bending stresses distant from the cusps.

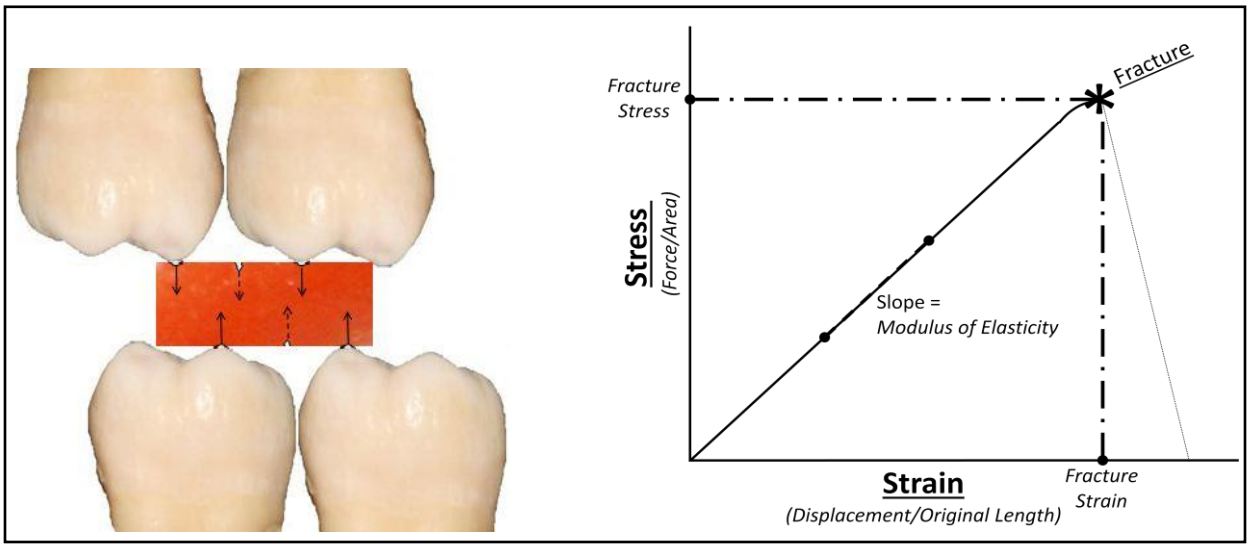

Figure 2.1. *Left: A model of food fracture between occluding teeth. Fractures can form as a result of indention at the cusp tips (solid arrows) or from tension away from the cusps (hatch-mark arrows). Right: A typical linear stress-strain curve. As the molars bite onto a food item, they apply a stress (force/area) that induces the material to strain (proportional deformation). This ultimately leads to the production of a crack in the material. The slope of the stress-strain curve is the food's modulus of elasticity, which is a measure of stiffness.*

Stress and strain (where strain is the proportional change in a dimension measured in a specified direction) at fracture are not true material properties because they depend on the size of the food object: a larger piece of food will break at lower stresses and strains than a smaller object of identical material composition. Both the stress and strain at failure do, however, provide comparative data of merit on the conditions of fracture for food items at similar particle sizes. In the case of food

processing methods that reduce ingested particle size (i.e. slicing and tenderizing), even though the fracture force per particle surface area may be higher, smaller individual particles will likely require less force and work to cleave because of their size (Kohyama et al., 2004b; Xu et al., 2008).

The slope of the stress-strain curve defines the modulus of elasticity, with higher slopes indicating a stiffer material. These curves are generally linear, but many foods have r-shaped stress-strain curves where the ‘r’ denotes a stress-strain curve that is concave downwards. This is typical of stiff foods that require disproportionate stress, compared to strain, towards fracture. More pliant foods have stress-strain curves that are concave upwards, giving them a 'j' shape (Purslow, 1991b; Sui et al., 2006), and typically requiring more strain to fracture. This is especially pertinent to the study of oral food fracture because of the limited space between occluding teeth, which means that more elastic foods may not fracture as readily. It is important to note that particularly for foods with 'j' shaped stress-strain curves, as the strain to failure approaches 0.5 or more, the definition of both stress and strain needs to change from its usual basis in the original dimensions of the object to those at the point of measurement (Ashby and Jones, 1996).

The property that controls the progress of a fracture is toughness, quantified here as the amount of work necessary to grow a crack of a given area after a fracture has already been initiated. Since most food objects show inherent flaws and weaknesses prior to being processed either orally or manually, toughness is actually the dominant measure of fracture force resistance in foods (and in a wide range of engineering materials (Ennos, 2012)).

A final food property of importance is energy dissipation. Stiff food objects, such as thick and turgid tubers, are likely to fracture in bending away from cusps, but softer ones will deform and mold themselves to the working surface of the teeth (Figure 2.1). In these cases, the only option for fracture inside the mouth is cuspal penetration. However, many softer, tougher foods dissipate energy internally making it impossible to ‘run’ a crack ahead of a cusp. Thus, upper and lower teeth must eventually meet

if there is to be a chance for food particle size reduction. The ability of foods to dissipate energy can be measured as the strain energy lost in viscous/plastic behavior, which is the area within the hysteresis loop during loading-unloading cycles (Oyen-Tiesma and Cook, 2001). This is particularly important to evaluate for animal soft tissues such as meat, and may help to predict the probability of fracture in the oral cavity; less energy dissipation should result in more efficient comminution (increased food breakdown per chew), fewer chews and reduced total masticatory force.

Hypotheses

Tubers. Mechanical tenderization by pounding with an Oldowan hammerstone will fracture and damage the internal cellular structure of tubers. Fractures in raw vegetables tend to burst cells, which is facilitated by internal turgor pressure that pre-stresses the cell walls (Ng and Waldron, 1997; Thiel and Donald, 2000; Lillford, 2001). The elastic modulus of cell walls themselves exceeds that of intact turgid vegetable tissue by a substantial margin and thus 'tenderized' vegetables may be stiffer, but the extensive fractures produced by a hammerstone will reduce overall toughness.

In terms of cooking, at temperatures above 40°C pectin substances are hydrolyzed and intercellular adhesion is reduced (Greve et al., 1994a; Ng and Waldron, 1997; Lillford, 2001). Thus, cooking will separate cells, with fluid invading intercellular spaces (Greve et al., 1994b; Thiel and Donald, 2000; Lillford, 2001). This will make roasted tubers more compliant, lowering the elastic modulus. Relaxed cell-cell bonds and lower turgor pressure cause fractures in cooked vegetables to run between cells instead of through them, thereby reducing fracture resistance (Ng and Waldron, 1997; Lillford, 2001). Therefore, roasting tubers should reduce both toughness and fracture stress. Additionally, cooking gelatinizes starch (Alvarez and Canet, 2001) and in higher starch tubers this may further decrease fracture resistance if starch granules lie in the fracture path.

Meat. Muscle tissue is approximately 75% water and 20% protein, with the majority (~85%) of the protein component made up of contractile muscle fibers (myofibrillar and sacroplasmic proteins). The remaining protein constituent is connective tissue, which surrounds muscle fibers and hierarchically groups them into longitudinal bundles (Tornberg, 2005). Disruption of the perimysium surrounding fiber bundles is probably the most important factor for oral processing (Purslow, 1991a). Pounding with an Oldowan hammerstone will mechanically disorganize the uniform arrangement of the fiber bundles and may also break the fibers themselves. Tenderizing in this manner should reduce both the strength and toughness of meat. The effect of mechanical tenderization of meat is predicted to be less than that for tubers because meat cells are not under turgor. Raw meat is extremely difficult to break into multiple pieces without a sharp cutting edge and it is likely that pounding with an Oldowan hammerstone will not fracture the meat efficiently.

Heat denatures (unfolds) and degrades muscle proteins, damages cell membranes and shrinks (dehydrates) the muscle fibers (Lewis and Purslow, 1989; Tornberg, 2005). Tensile strength of meat heated in a water bath increases in two stages, from ~40-50°C and then again from 60-90°C (Christensen et al., 2000). Changes below 60°C are primarily caused by the denatured connective tissue; collagen straightens and more fibers are packed into the same volume, increasing overall strength (Lewis and Purslow, 1989; Christensen et al., 2000). At 50°C, the collagen solubilizes, and connective tissue strength progressively decreases (Christensen et al., 2000). Additionally, sarcoplasmic proteins form aggregates between ~50-60°C, which may decrease the elastic crack blunting properties of raw muscle and result in less fracture stress in this temperature range (Tornberg, 2005). Increased meat strength above 60°C is driven by muscle fiber denaturation and possibly shrinkage and water loss, which increases protein concentration (Christensen et al., 2000; Tornberg, 2005). The conformation and chemical changes from heating also increase muscle stiffness (Lewis and Purslow, 1989; Tornberg, 2005) and fracture strain (Willems and Purslow, 1997). Taken together, these well documented effects lead to the prediction that

dry roasting meat will also increase toughness, fracture stress and strain, and stiffness. Increased evaporation from open air cooking may further promote heat-related water loss and shrinkage, leading to an even stiffer and more fracture resistant material. Stiffer meat with roasting will also likely reduce elastic energy loss. If roasted meat becomes extremely stiff (i.e., brittle), fractures will propagate more efficiently and toughness may decrease because less external work is required.

Mechanical Processing. Although dental size was variable, postcanine crown area was approximately 21-25% smaller in *H. erectus* than in *H. habilis* and gracile australopiths, respectively, and *H. sapiens* molars and premolars were a further 15% smaller (Brace, 1967; Wolpoff, 1973; McHenry, 1994; McHenry and Coffing, 2000). In addition to hypotheses concerning specific material property changes, this study also tests the general hypothesis that mechanical processing of foods provides sufficient reductions in masticatory effort to explain the pronounced dental size reductions of *H. erectus* compared to *H. habilis* and gracile australopiths. Lucas (2004) models postcanine occlusal area reductions in terms of a predicted decrease in food toughness based on fracture scaling. Dependent on the fracture behavior of a food, as affected by tenderizing or cooking, it can be predicted that typical food toughness will scale with dental size to the power 0.5. This leads to the expectation that mechanical tenderization caused by pounding will reduce the average toughness of foods 38-44%, allowing for the roughly 21-25% reduction in postcanine occlusal area of *H. erectus* relative to *H. habilis* and gracile *Australopithecus* species. A further reduction in toughness on top of this (~27%) caused by cooking allowed for the approximately 15% smaller teeth of *H. sapiens*. If toughness does not decrease with mechanical tenderization of the foods, the argument for early adoption of cooking by *H. erectus* is supported.

METHODS

Materials.

Organic tubers, red beets (*B. vulgaris*), carrots (*D. carota*) and jewel yams (*I. batatas*), were purchased from a local store and stored at 4°C for no more than 4 days prior to processing and material property testing. For the meat portion of the experiment, an adult goat (*C. aegagrus*) was purchased from a local farm and slaughterhouse (Blood Farms, Groton, MA) and the fresh carcass transported on ice to the Skeletal Biology Lab, Harvard University. Muscle groups were removed using aseptic procedures, sealed in vacuum bags and stored at -20°C. Meat was defrosted at 4°C for approximately 12-24 hours prior to data collection.

Processing Procedure.

To limit material property variation caused by factors other than processing, each tuber was divided into two portions (one was kept raw, and the other was roasted or tenderized) and the same muscle regions were used for each meat experiment (neck muscle for toughness tests and knee flexors for the tensile tests).

Mechanical Processing (Tenderization): A replica Oldowan hammerstone was used to mechanically tenderize the food. The medulla of each tuber was sliced into 13 mm cubes, then each cube hit six times with the hammerstone. Goat was cut into a 50g steak and hit 20 times. This type of simple tenderization affected tubers and meat differently. The tubers fractured into numerous, relatively large pieces, while the meat remained intact but was significantly 'mashed' and the muscle fibers disorganized where struck by the hammerstone (Figure 2.2).

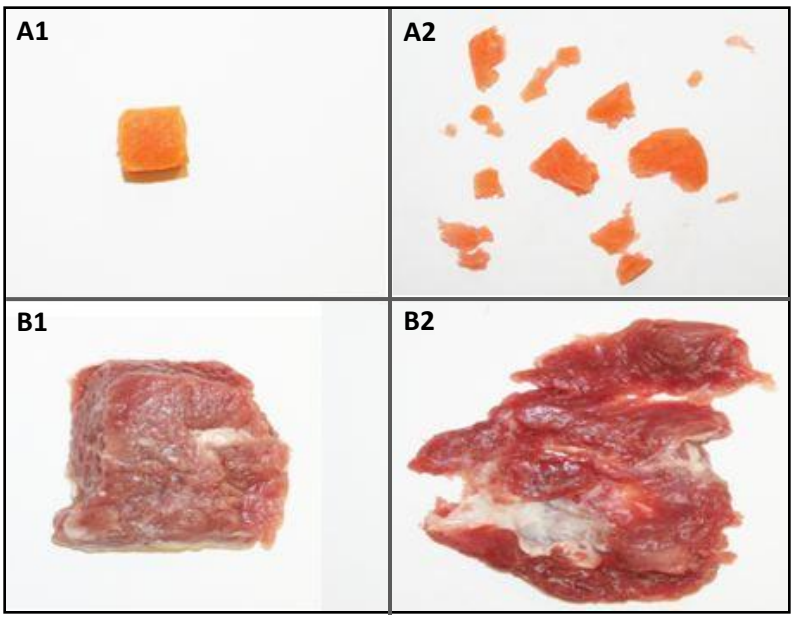

Figure 2.2. *A: Carrot before (A1) and after (A2) mechanical tenderization with a replica Oldowan hammerstone. The carrot was struck 6 times, breaking it into numerous smaller pieces. B: 50g goat steak before (B1) and after (B2) mechanical tenderization with a replica Oldowan hammerstone. The steak was struck 20 times, which primarily mashed and disorganized the muscle fibers; the steak remained in one piece.*

Roasting: Samples were roasted on a warmed-up, tabletop propane grill (Perfect Flame™) with the lid open and the gas flow valve set to "high". Although hominins would not have used grills, roasting in this manner is similar to an open fire (the flames are immediately below the grill surface) and allowed cooking of multiple samples at a consistent temperature. Internal temperatures were measured on selected samples immediately post-cooking (tubers) or during cooking (meat) with a needle probe and digital thermocouple (Thermoworks™, accuracy ± 0.1°C). Pre- and post-roasted weights were recorded (digital scale, accuracy ± 0.1g).

Before being roasted, each tuber was cut into 17 mm-thick transverse slices. The slices were then roasted for 15 minutes, 7.5 minutes on each side, with each slice rotated to a different spot every

2.5 minutes during the cooking process to ensure even heating despite grill surface temperature variations. This protocol heated beets to 78.6±2.2°C, carrots to 78.5±1.1°C, and yams to 89.0±2.7°C, and produced a 22±3%, 27±3% and 17±2% reduction in slice weight (i.e. water loss), respectively (Table 2.1).

Table 2.1. Weight change (water loss) and internal temperature of tubers roasted for 15 minutes.[a]

	Pre-Roasted Weight *(g)*	Post- Roasted Weight *(g)*	% Reduction	Internal Roasted Temperature *(°C)*[b]
Beet *(n=5)*	62.6	50.9	18.7	75.3
	72.2	58.0	19.7	78.9
	50.7	39.7	21.7	78.4
	46.5	34.4	26.0	79.2
	41.5	30.8	25.8	81.4
		Average (S.D.)	***22.4%** (3.4%)*	***78.6° C** (2.2°C)*
Carrot *(n=5)*	16.3	11.2	31.3	76.9
	13.4	9.9	26.1	78.2
	13.7	10.4	24.1	78.6
	12.4	9.1	26.6	79.8
	15.1	11.2	24.5	79.2
		Average (S.D.)	***26.8%** (2.7%)*	***78.5° C** (1.1° C)*
Yam *(n=5)*	58.7	49.5	15.7	87.5
	63.0	52.3	17.0	90.1
	50.1	41.1	18.0	93.3
	52.1	43.6	16.3	86.3
	58.3	46.6	20.1	88
		Average (S.D.)	***17.4%** (1.7%)*	***89.0° C** (2.7° C)*

[a] *Tubers were sliced to a uniform thickness (17 mm) and were roasted for 15 minutes on a table-top propane grill.*

[b] *Temperature was measured with a needle-probe thermocouple (Thermoworks™, accuracy ± 0.1°C) inserted into the center of each slice immediately after cooking.*

Two pieces of meat, one from the neck muscles (A) and the other from the knee flexors (B), were roasted on the center of the grill. They were periodically turned over and were cooked until 'well-done' and only slightly pink internally (internal temperature, A = 72.2°C, B = 76.0°C). The two steaks

differed greatly in size (A = 67.7g, B = 331.7g) and the larger took almost twice as long to reach 'well-done' (A = 24 minutes, B = 46 minutes). Water loss was similar for both steaks (A = 35%, B = 40%).

Material Property Testing.

A Darvell™ HKU portable mechanical property tester (Darvell et al., 1996) and an Instron™ 5564 were used to measure the material properties of the food samples. Tester attachments were cleaned with alcohol between each trial. Unless noted, ten samples of each raw, mechanically processed and roasted food type were tested. Toughness was the only property measured for tenderized tubers and meat because the processing procedure produced small, highly fragmented pieces that precluded additional testing. Each sample was measured with digital or dial (meat tensile tests only) calipers (accuracy, ±0.01 and ±0.02 mm, respectively). Raw and roasted meat samples were tested both parallel and perpendicular to fiber direction. Because tuber material properties can change drastically depending on the region of the food sampled, testing was only performed on the inner region (medulla). Samples were wrapped in damp paper towels and stored in plastic bags at 4°C until immediately before testing.

Toughness (Tubers): Two experiments were performed, one testing the effect of roasting and the other testing the effect of mechanical tenderization. Toughness was measured using a 15° included angle wedge fitted onto the Darvell tester (100N load cell). The wedge allows for controlled crack growth in semi-brittle foods, such as tubers (Vincent et al., 1991).

Experiment #1. Raw and roasted tuber samples were cut into rectangular blocks approximately 12-14 mm wide. A wedge was lowered into each sample and stopped when it just slightly pierced the material. At this point, the wedge was continuously lowered at a rate of ~30mm/min, while recording the force (F) and resulting displacement (u) every 0.001mm, for a final slice depth of 5 mm. The wedge was removed from the sample and reinserted into the previously formed crack to measure the work not

used in crack formation (i.e. work due to friction and elastic bending of material against the crack faces). This work was subtracted from the original work value. Crack width (w) was measured after testing and toughness for each sample was calculated for a 2 mm crack depth (between 2-4 mm, determined by wedge displacement).

Experiment #2. Experimental set-up and toughness calculations were the same as experiment #1. The only differences were that the samples were not a uniform size and data collection began after the wedge was completely inserted into the sample (samples were the larger of the pieces resulting from the tenderization process and the tops were irregular). Additionally, because they were smaller than the raw samples, maximum wedge depth was 1.5 mm and the toughness for each tenderized piece was calculated between a depth of 0.3 and 1.3 mm (i.e. crack depth, u = 1 mm).

- Toughness = (Work $_{initial\ pass}$ – Work $_{second\ pass}$) / w u

Toughness (Meat): Meat toughness was measured using Swissors®, a pair of tailoring scissors, fitted onto the Darvell tester (100N load cell). Cutting tests enable measurements of controlled crack growth in floppy (high elastic) materials such as meat (Atkins and Mai, 1979; Lucas et al., 2001).

Using a bacon slicer, strips of raw and roasted meat of an even 2-4 mm thickness, and 12-16 mm in width, were cut. Tenderized samples were trimmed using a razor blade and gently formed into similar sized strips. Each sample was placed between the scissor blades, and the handle depressed at a displacement rate of ~30mm/min, while recording the force (F) and resulting displacement (u) every 0.001 mm until the sample was completely cut. The thickness (t) and length (l) of each cut was measured and the work required to make the cut calculated. Work due to friction was calculated by closing the scissors with no sample between the blades. This work was then was subtracted from the original work value to calculate toughness.

- Toughness = (Work $_{initial\ pass}$ – Work $_{second\ pass}$) / t l

Compression Tests (Tubers): A cork borer was used to cut uniform cylinders (radius ~3 mm, height ~8mm) from the center of each tuber, parallel to the long axis of the root. The radius (r) and length (l_o) of each sample was recorded. The modulus of elasticity and fracture stress and strain were then recorded by continuously compressing each sample between two plates (displacement rate ~32 mm/min) on the Darvell tester (1000N load cell), recording the force (F) - displacement (u) relationship every 0.001 mm.

The data were then converted into true stress and strain by assuming conservation of volume (i.e., a Poisson's ratio of 0.5, a common assumption in food analysis and supported by measurements such as by Finney and Hall (1967) on potato). 'True' stress and strain were necessary because as failure strain approaches 0.5 or more, the definition of both stress and strain needs to change from its usual basis in the original dimensions of the object to those at the point of measurement (Ashby and Jones, 1996). Strain was then converted to absolute measures; a negative value obtained in the calculation (below) signifies a compressive state. Peak stress (fracture stress) and the corresponding strain were recorded, and the modulus of elasticity (slope of the line) was calculated at 20%, 40%, 60% and 80% fracture stress. Multiple measures are needed because a linear stress-strain relationship is not anticipated for all foods.

- Stress = $F(l_o - u) / \pi r^2 l_o$
- Strain = $\ln((l_o - u)/l_o)$

Tensile Tests (Meat): A bacon slicer was used to cut uniform samples of raw and roasted meat. The thickness (t) and width (w) of each strip (approximately 2 mm and 11 mm, respectively) were recorded. Samples were then placed into an Instron tester (50N load cell) using two pneumatic grips 30 mm apart (l_o) and increasingly loaded in tension (displacement rate 90mm/min). Two sets of

experiments were performed, a fracture test and a cycling test. Force (F) and displacement (u) were recorded every 0.05 s for the fracture test and 0.034 s for cycling trials.

Experiment #1. Each sample was loaded until the meat had completely fractured into two. As for tubers, the force-displacement data was converted into true stress and strain by assuming the incompressibility of muscle in its passive state (Van Loocke et al., 2006). Fracture stress, fracture strain and the modulus of elasticity at 20%, 40%, 60% and 80% fracture stress were recorded. One sample (raw meat, force parallel to fiber direction) did not completely fracture and was omitted from analyses.

- Stress = $F(l_o + u) / t\ w\ l_o$
- Strain = $\ln((l_o + u)/l_o)$

Experiment #2. Each sample was cycled to a peak strain of 4%, 8%, 12% and 16%, and unloaded back to the initial position between each successively large strain loop. Fractures did not initiate during these trials. The percent work lost on unloading for each loop was calculated. Five samples of each food type (raw and roasted, parallel and perpendicular to muscle fiber direction) were tested.

Analyses.

All calculations were performed in Excel (Microsoft 2007), StatView statistical package (SAS Institute, version 5.0.1), and custom written programs in LabView 8.1. In order to avoid assumptions of normal distributions in the data, Mann-Whitney U tests were used to compare the raw and processed food values. Significance was set at $p \leq 0.05$.

RESULTS

Toughness. (Table 2.2)

Roasting reduced the toughness of tubers by 49%, from an average of 1034±345 J/m^2 (raw) to 526±120 J/m^2 (roasted) ($p<0.0001$) (Figure 2.3). Mechanically tenderizing the tubers produced a similar result; toughness decreased 42% from 1080±167 J/m^2 (raw) to 622± 297 J/m^2 (tenderized) ($p<0.0001$) (Figure 2.4). Tenderization reduced beet toughness more than roasting (tenderization = 65%; roasting = 56%), while the converse was true for yams (roasting = 48%; tenderization = 28%). The percent decrease in carrot toughness was the same regardless of whether it is roasted (39%) or tenderized (38%).

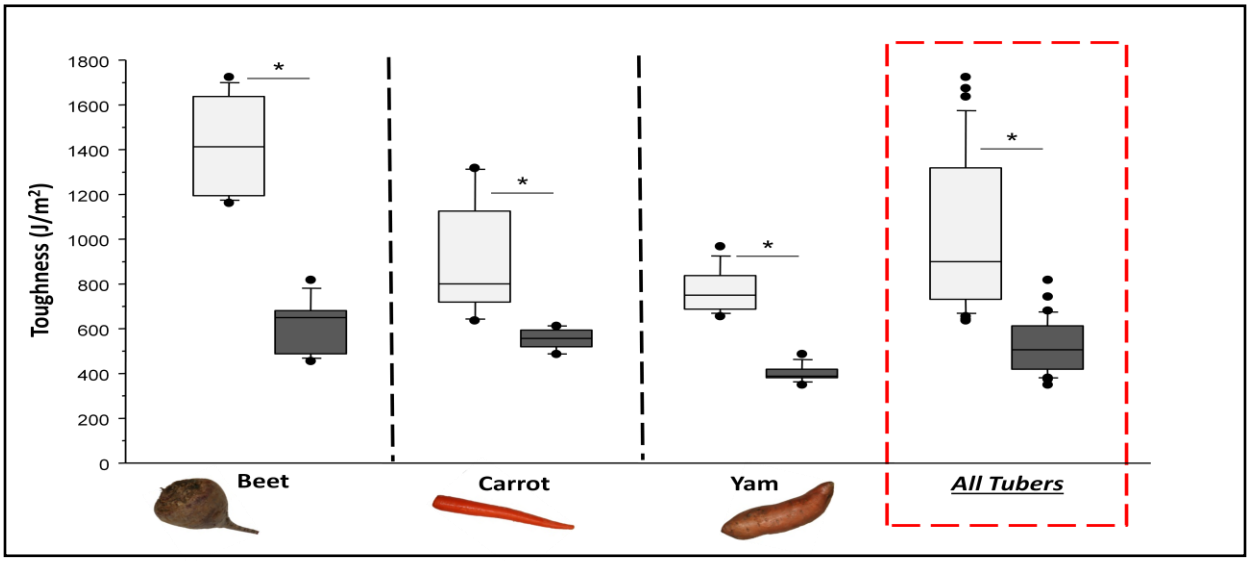

Figure 2.3. *Toughness (J/m^2) of raw (light) and roasted (dark) beets, carrots and yams, with the pooled - tuber average shown at right. Box plot whiskers extend to the 10th and 90th percentiles. *$p \leq 0.05$, Mann-Whitney U Test.*

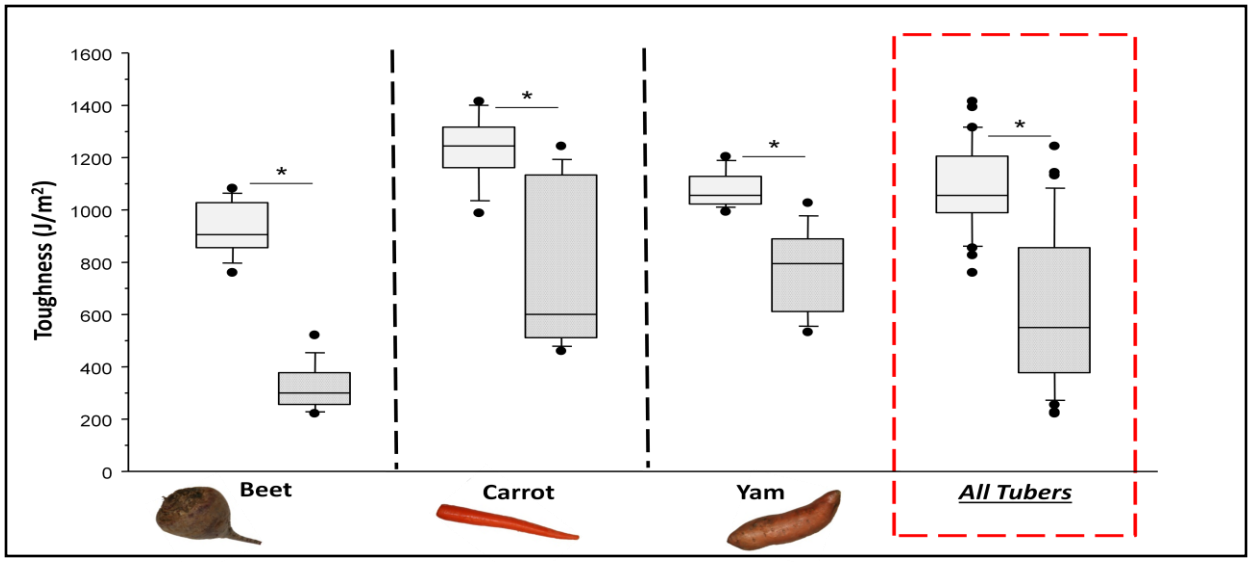

Figure 2.4. *Toughness (J/m^2) of raw (light) and tenderized (hatch-mark) beets, carrots and yams, with the pooled -tuber average shown at right. Box plot whiskers extend to the 10th and 90th percentiles. *p ≤ 0.05, Mann-Whitney U Test.*

In contrast to the results for tubers, roasting increased the toughness of meat by 77% (raw average = 216±78 J/m^2; roasted average = 381±180 J/m^2; p<0.01) (Figure 2.5). Toughness varied greatly depending on whether it was measured parallel or perpendicular to fiber direction. The former primarily measures the toughness of the weaker connective tissues sheaths surrounding the fibers, while the latter is a measure of the tougher muscle fibers themselves and although roasting increased both of these values (raw parallel = 154±47 J/m^2; roasted parallel = 218±49 J/m^2; p=0.01) (raw perpendicular = 277±46 J/m^2; roasted perpendicular = 545±84 J/m^2; p<0.001), its effect was greatest perpendicular to fiber direction (perpendicular = 97% increase; parallel = 41% increase).

Unlike with tubers, tenderizing did not affect meat toughness (Figure 2.5). Although tenderized meat (163±68 J/m^2) was less tough than raw meat measured across the muscle fibers (p<0.01), it did not differ significantly from the toughness of raw meat measured parallel to fiber direction or average raw

meat toughness (average raw meat toughness parallel and perpendicular to fiber direction, 216±78 J/m^2).

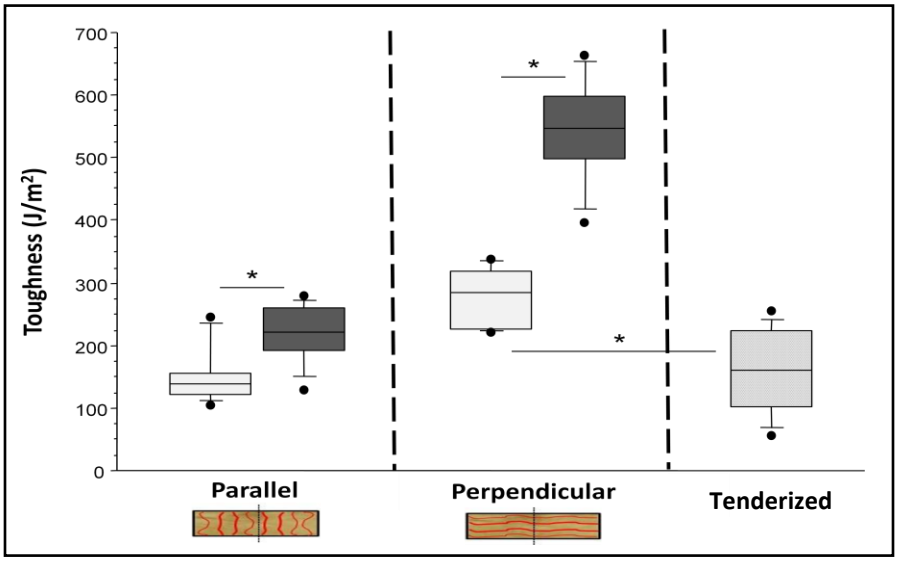

Figure 2.5. *Toughness (J/m^2) of raw (light), roasted (dark), and tenderized (hatch-mark) meat. Toughness of raw and roasted meat was measured both parallel and perpendicular to muscle fiber direction. Tenderized meat was compared to the two raw meat samples. Box plot whiskers extend to the 10^{th} and 90^{th} percentiles. *$p \leq 0.05$, Mann-Whitney U Test.*

Table 2.2. Average toughness (J/m^2) of raw, roasted and tenderized tubers and goat meat.[a]

			Treatment	Toughness *(J/m^2)*[b]
TUBERS	Experiment #1	Beet *(n=10)*	*Raw*	1424.9 (208.8)
			Roasted	622.6 (120.0)
		Carrot *(n=10)*	*Raw*	903.8 (256.0)
			Roasted	553.0 (48.1)
		Yam *(n=10)*	*Raw*	774.6 (101.3)
			Roasted	403.0 (39.4)
		Tuber Average *(n=30)*	*Raw*	1034.4 (344.7)
			Roasted	526.2 (119.8)
	Experiment #2	Beet *(n=10)*	*Raw*	922.7 (103.5)
			Tenderized	322.2 (90.5)
		Carrot *(n=10)*	*Raw*	1239.7 (134.0)
			Tenderized	769.1 (317.1)
		Yam *(n=10)*	*Raw*	1078.5 (71.3)
			Tenderized	773.6 (162.3)
		Tuber Average *(n=30)*	*Raw*	1080.3 (166.8)
			Tenderized	621.6 (297.1)
MEAT[c]	Parallel *(n=10)*		*Raw*	154.3 (46.5)
			Roasted	218.2 (48.7)
	Perpendicular *(n=10)*		*Raw*	277.1 (46.3)
			Roasted	544.6 (84.2)
	Tenderized (n=10)			163.3 (68.0)

[a] *One standard deviation is in parentheses. See text for food processing details.*

[b] *Toughness of tubers and meat was measured using wedge and cutting tests, respectively.*

[c] *Meat was measured both parallel and perpendicular to muscle fiber direction.*

Tuber Compression Tests. (Table 2.3)

With one exception (noted below), roasting significantly affected the fracture stress, fracture strain and stiffness of tubers. In all cases, yams were more altered by roasting than carrots and beets (average measurement change was 74%).

The stress required to fracture tubers was greatly lowered with roasting, decreasing 28% from a raw average of 1349±349 kPa to a roasted average of 974±555 kPa ($p<0.01$) (Figure 2.6). This reduction was greatest for yams (69%), followed by carrots (20%), and beets (11%). Conversely, the average compressive strain required for fracture increased by 58%, going from 0.33±0.09 in raw tubers to 0.52±0.19 with roasting ($p<0.0001$) (Figure 2.7). As with fracture stress, the greatest effect was for yams (74%), although beets followed closely (70%). Carrot fracture strain, in comparison, increased a modest 34% with roasting.

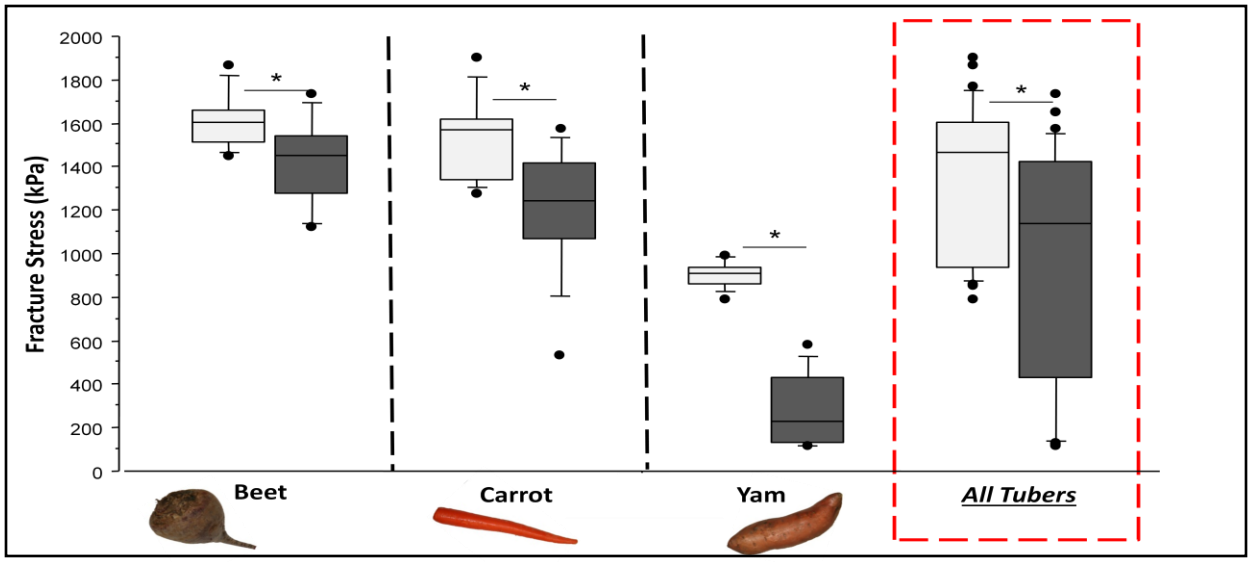

Figure 2.6. *Fracture stress (kPa) of raw (light) and roasted (dark) beets, carrots and yams, and the average of the three tubers (red hatch-mark box). Box plot whiskers extend to the 10th and 90th percentiles. *$p \leq 0.05$, Mann-Whitney U Test.*

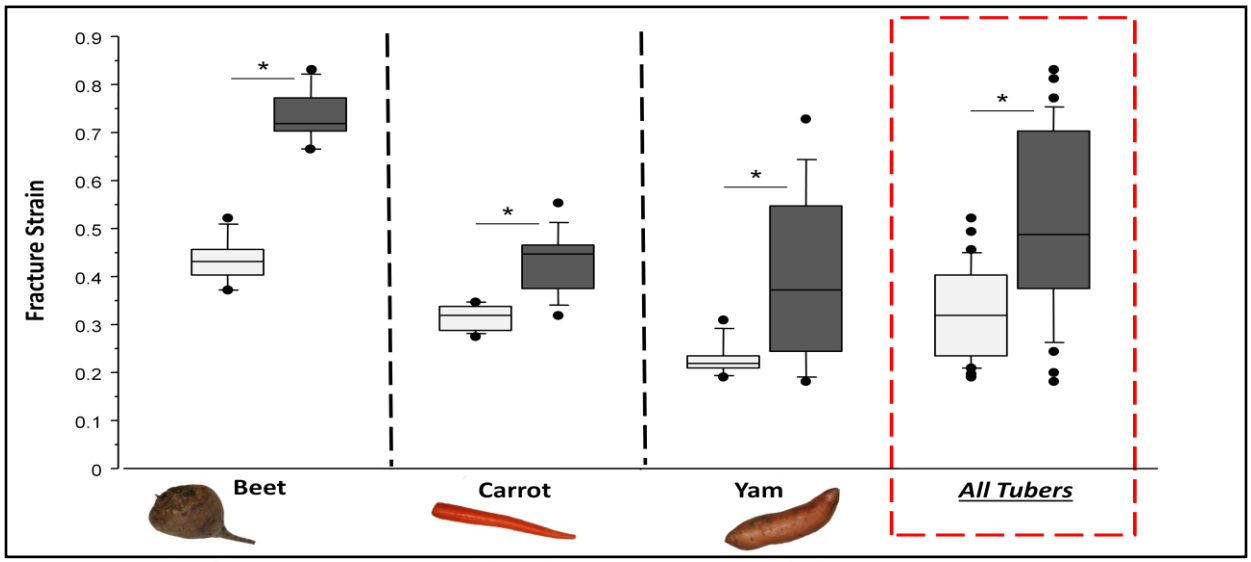

Figure 2.7. *Fracture strain (kPa) of raw (light) and roasted (dark) beets, carrots and yams, and the average of the three tubers (red hatch-mark box). Box plot whiskers extend to the 10th and 90th percentiles. *p ≤ 0.05, Mann-Whitney U Test.*

When stress-strain curves for raw and roasted tubers are plotted, the curves change in shape from linear/slightly r-shaped (raw) to J- or even slightly s-shaped (roasted) (Figure 2.8). Comparing modulus values, roasting decreased the stiffness of all tubers (45% reduction, tuber and modulus average; p>0.0001), with the exception of carrots measured at 40% fracture stress (no significant difference) (Figure 2.9). Average raw tuber modulus started off high at 6026±2087 kPa (measured at 20% fracture stress) and declined steadily to 4627±1095 kPa (measured at 80% fracture stress). In contrast, roasted tubers followed a different pattern, starting off at a low of 2449±1332 kPa and then increasing to a high of 3174±1582 kPa, before decreasing to 3024±1535 kPa and 2629±1491 kPa (measured at 20%, 40%, 60% and 80% fracture stress, respectively). Yams had the greatest reduction in overall stiffness with roasting (76%), followed by carrots (31%) and beets (30%).

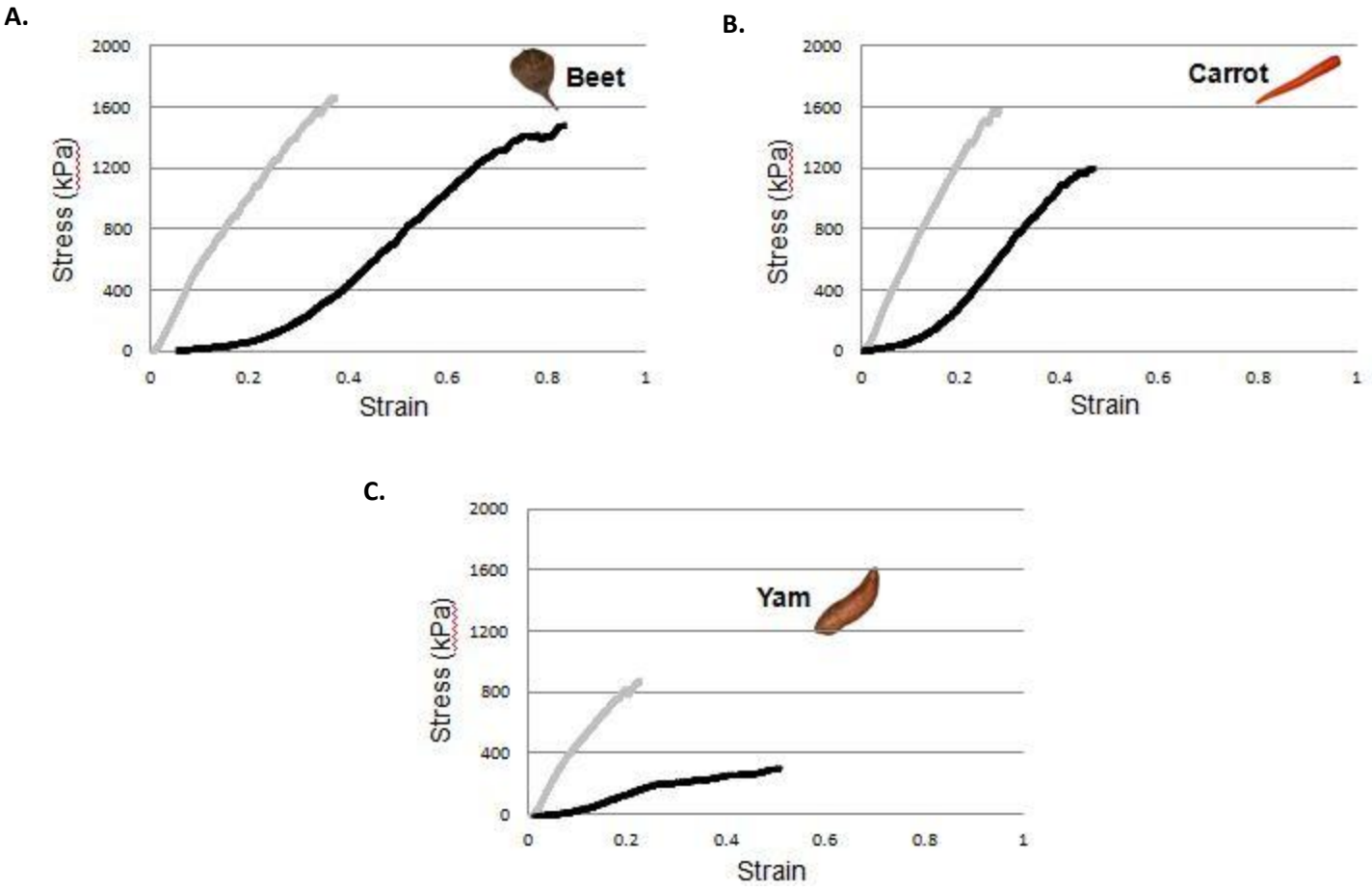

Figure 2.8. *Representative stress-strain curves of raw (light) and roasted (dark) beets, carrots and yam. Fracture occurred at the last point displayed.*

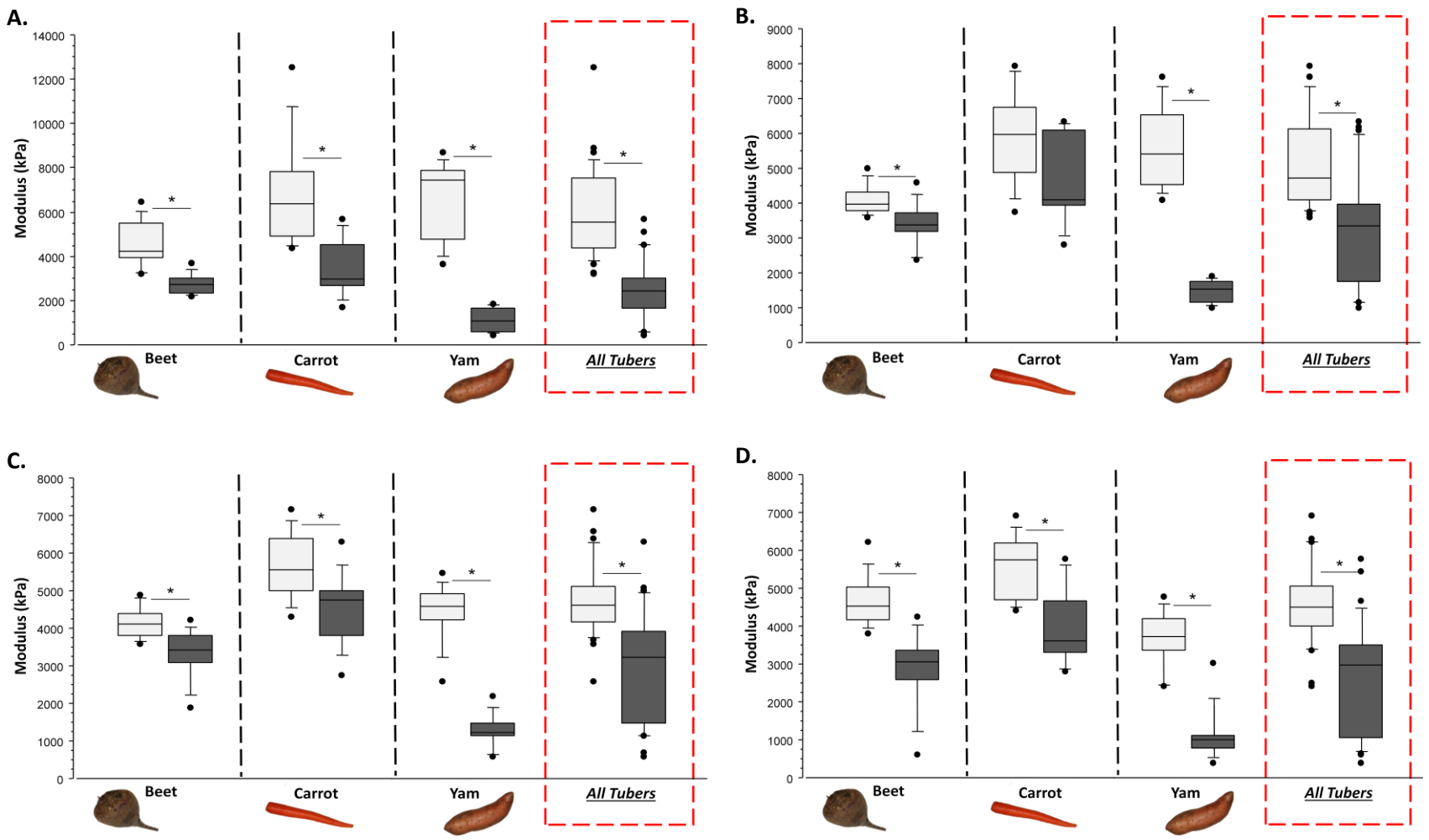

Figure 2.9. *Elastic modulus (kPa) of raw (light) and roasted (dark) beets, carrots and yams, and the average of the three tubers (red hatch-mark box). Modulus was measured at 20% (A), 40% (B), 60% (C), and 80% (D) fracture stress. Box plot whiskers extend to the 10th and 90th percentiles. * $p \leq 0.05$, Mann-Whitney U Test.*

Table 2.3. Average fracture stress (kPa) and strain and modulus of elasticity (kPa) of raw and roasted tubers and goat meat.[ab]

		Treatment	Fracture Stress *(kPa)*	Fracture Strain	Modulus 20% Fracture Stress *(kPa)*[c]	Modulus 40% Fracture Stress *(kPa)*[c]	Modulus 60% Fracture Stress *(kPa)*[c]	Modulus 80% Fracture Stress *(kPa)*[c]
TUBERS (Experiment #1)	**Beet** *(n=10)*	*Raw*	1610.1 (130.8)	0.43 (0.05)	4579.0 (1076.3)	4090.3 (439.2)	4159.6 (431.3)	4661.9 (692.0)
		Roasted	1427.7 (201.0)	0.73 (0.06)	2737.8 (461.3)	3385.3 (646.0)	3311.6 (690.0)	2843.2 (1028.1)
	Carrot *(n=10)*	*Raw*	1531.1 (199.7)	0.32 (0.03)	6816.4 (2520.0)	5982.8 (1328.4)	5659.7 (907.3)	5570.5 (866.2)
		Roasted	1217.8 (299.1)	0.43 (0.07)	3527.7 (1326.8)	4674.0 (1316.7)	4498.5 (976.7)	3943.0 (1019.1)
	Yam *(n=10)*	*Raw*	905.8 (59.0)	0.23 (0.04)	6682.3 (1740.2)	5583.7 (1237.2)	4411.7 (785.0)	3649.8 (764.9)
		Roasted	277.1 (166.2)	0.40 (0.18)	1081.2 (525.6)	1463.9 (337.4)	1262.9 (455.9)	1102.0 (713.6)
	Tuber Average *(n=30)*	*Raw*	1349.0 (348.5)	0.33 (0.09)	6025.9 (2087.1)	5218.9 (1330.0)	4743.7 (974.4)	4627.4 (1095.3)
		Roasted	974.2 (554.8)	0.52 (0.19)	2448.9 (1331.6)	3174.4 (1581.9)	3024.4 (1534.9)	2629.4 (1491.2)
MEAT[d]	**Parallel** *(n=10)*[e]	*Raw*	96.0 (32.4)	0.65 (0.16)	148.6 (65.4)	148.3 (47.4)	246.5 (59.7)	328.8 (106.7)
		Roasted	309.2 (116.6)	0.48 (0.20)	1066.7 (580.1)	935.7 (556.2)	822.4 (369.6)	733.6 (166.9)
	Perp. *(n=10)*	*Raw*	20.3 (9.8)	0.54 (0.11)	53.1 (41.3)	66.1 (50.0)	65.7 (52.7)	78.6 (45.6)
		Roasted	30.7 (8.9)	0.49 (0.12)	55.8 (20.6)	90.6 (25.3)	84.6 (40.5)	111.8 (39.4)

[a] *One standard deviation is in parentheses. See text for food processing details.*

[b] *Properties of tubers and meat were measured using compression and tensile tests, respectively.*

[c] *Elastic modulus was measured at 20%, 40%, 60% and 80% fracture stress.*

[d] *Meat was measured both parallel and perpendicular (perp.) to muscle fiber direction.*

[e] *One raw meat sample (measured parallel to fiber direction) did not completely fracture and was omitted from analyses, reducing sample size to nine.*

Meat Tensile Tests. (Table 2.3)

Unlike in tubers, roasting increased the fracture stress of meat, with the greatest change occurring parallel to fiber direction (Figure 2.10). Maximum stress at fracture went from 20±10 kPa (raw) to 31±9 kPa (roasted), when meat was pulled perpendicular to the direction of the fibers, primarily breaking the weaker connective tissue rather than the fibers themselves (p=0.03). When meat was tensed parallel to fiber direction, fracture stress rose from 96±32 kPa (raw) to 309±117 kPa (roasted), a 222% increase (p<0.001). The maximum amount of tensile deformation required to fracture the meat into two separate pieces did not significantly change with roasting (raw parallel = 0.65±0.16; roasted parallel = 0.48±0.20) (raw perpendicular = 0.54±0.11; roasted perpendicular = 0.49±0.12) (Figure 2.11).

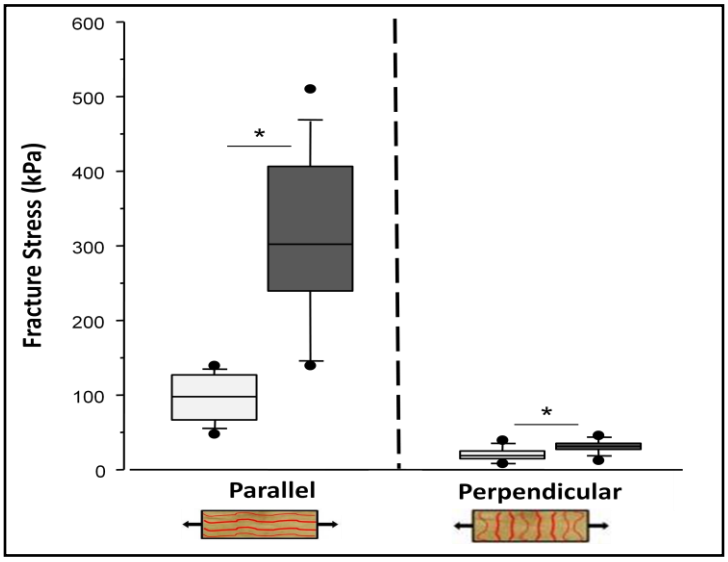

Figure 2.10. *Fracture stress (kPa) of raw (light) and roasted (dark) meat. Meat was loaded in tension both parallel and perpendicular to muscle fiber direction. Box plot whiskers extend to the 10th and 90th percentiles. * p ≤ 0.05, Mann-Whitney U Test.*

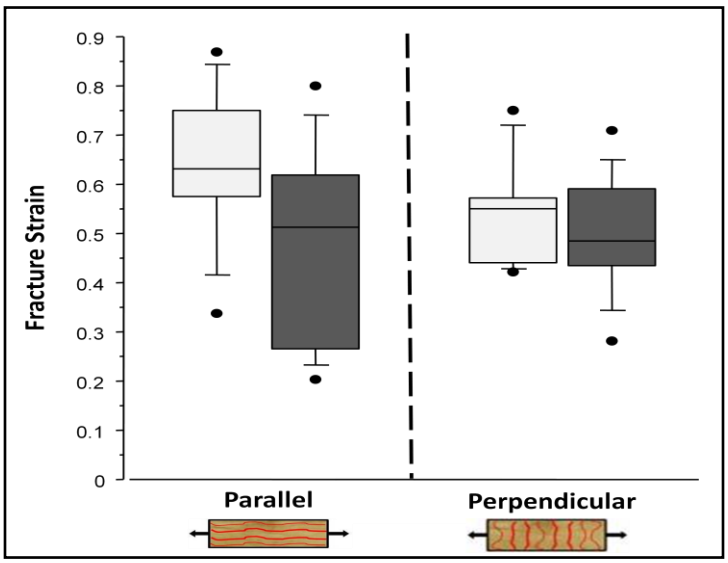

Figure 2.11. *Fracture strain of raw (light) and roasted (dark) meat. Meat was loaded in tension both parallel and perpendicular to muscle fiber direction. Box plot whiskers extend to the 10th and 90th percentiles. * $p \leq 0.05$, Mann-Whitney U Test.*

Roasting changed the shape of the stress-strain curve from linear or slightly J-shaped to r-shaped, but only when pulling parallel to fiber direction (Figure 2.12). Curve shape remained the same (J-shaped) when meat was pulled perpendicular to fiber direction, regardless of whether it was raw or roasted. A similar pattern emerges for comparisons of stiffness (Figure 2.13). The average modulus of roasted meat, parallel to fiber direction, was 308% higher than raw meat ($p<0.001$), while the modulus measured perpendicular to fiber direction did not significantly change. Parallel to fiber direction, the raw meat modulus was unchanged at 20% and 40% fracture stress (149±65 kPa and 148±47 kPa, respectively), and then increased to 247±60 kPa and 329±107 kPa (measured at 60% and 80% fracture stress, respectively). Comparable roasted meat modulus, however, steadily decreased with increasing fracture stress, going from a high of 1067±580 kPa to 936±556 kPa, 822±370 kPa and 734±167 kPa (measured at 20%, 40%, 60% and 80% fracture stress, respectively). Regardless of whether the meat was

raw or roasted, stiffness perpendicular to the direction of the fibers was much lower than when measured parallel to fiber direction (Raw = perpendicular 70% lower than parallel; Roasted = perpendicular 90% lower than parallel; $p<0.001$).

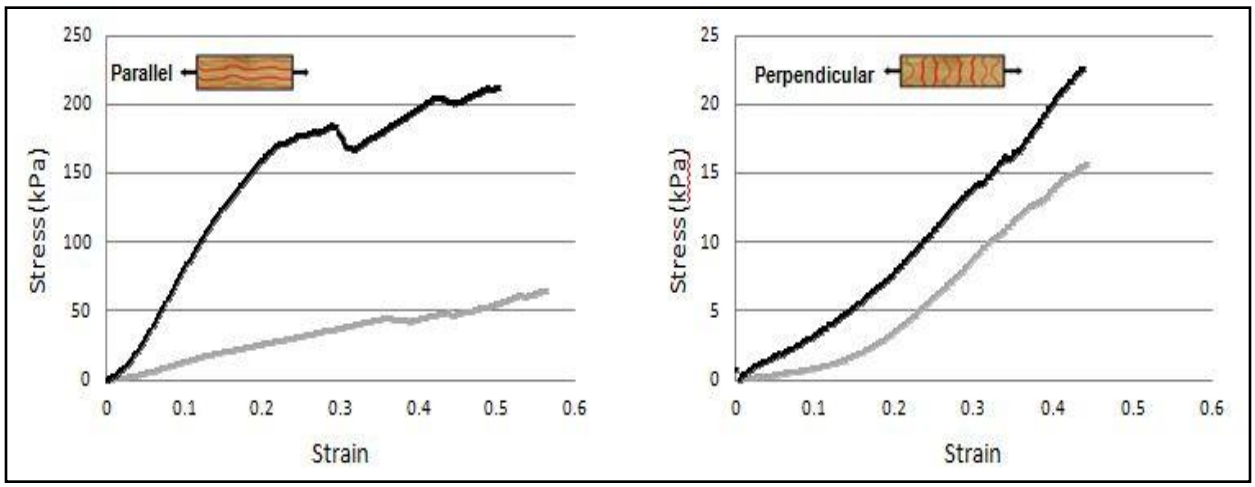

Figure 2.12. *Average stress-strain curves of raw (light) and roasted (dark) meat. Meat was loaded in tension both parallel and perpendicular to muscle fiber direction.*

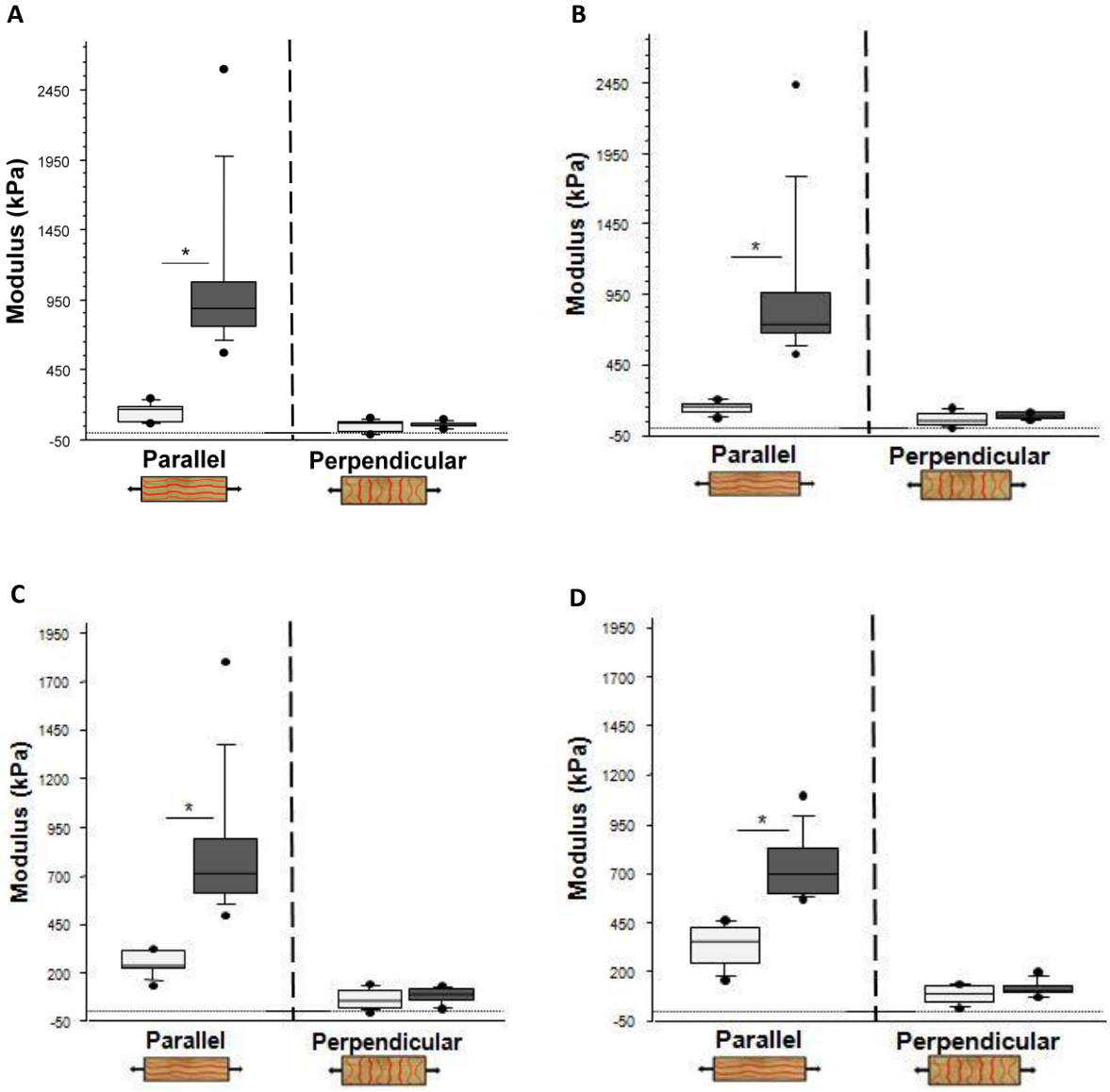

Figure 2.13. *Elastic modulus (kPa) of raw (light) and roasted (dark) meat loaded in tension both parallel and perpendicular to muscle fiber direction, measured at 20% (A), 40% (B), 60% (C), and 80% (D) of the fracture stress. Box plot whiskers extend to the 10th and 90th percentiles. *p ≤ 0.05, Mann-Whitney U Test.*

Meat Cycling. (Table 4)

Roasting significantly decreased work loss (i.e. energy dissipation), but only when cycling parallel to fiber direction ($p<0.01$) (Figure 2.14). On average, roasted samples lost 28% less energy than raw samples (66±11% vs. 38±3%, 74±11% vs. 44±4%, 77±10% vs. 49±3%, 78±9% vs. 51±3% for raw vs. roasted samples tensed to 4%, 8%, 12% and 16% strain, respectively). There was no significant difference in energy loss between raw and roasted samples pulled perpendicular to fiber direction. Although roasting had no effect, average energy loss measured in this direction was relatively low and comparable to that of roasted meat measured parallel to fiber direction (45±7% versus 45±6%, respectively).

Table 2.4. Average percent work lost (energy dissipation) during loading-unloading cycles of raw and roasted meat. [ab]

		PARALLEL *(n=5)*		**PERPENDICULAR** *(n=5)*	
		Raw	**Roasted**	**Raw**	**Roasted**
4% Strain	Work (J), Loaded	7.03×10^{-5} (2.23×10^{-5})	2.34×10^{-4} (1.21×10^{-4})	1.50×10^{-5} (9.99×10^{-6})	3.27×10^{-5} (1.26×10^{-5})
	Work Lost (J), Unloaded	4.62×10^{-5} (1.77×10^{-5})	8.87×10^{-5} (4.64×10^{-5})	6.21×10^{-6} (3.49×10^{-6})	1.44×10^{-5} (6.68×10^{-6})
	% Work Lost [c]	65.6% (11.3%)	37.9% (2.5%)	43.6% (6.8%)	43.4% (7.6%)
8% Strain	Work (J), Loaded	2.46×10^{-4} (6.59×10^{-5})	1.02×10^{-3} (4.93×10^{-4})	6.42×10^{-5} (4.73×10^{-5})	1.11×10^{-4} (3.76×10^{-5})
	Work Lost (J), Unloaded	1.79×10^{-4} (4.73×10^{-5})	4.57×10^{-4} (2.26×10^{-4})	2.60×10^{-5} (2.41×10^{-5})	5.00×10^{-5} (2.35×10^{-5})
	% Work Lost [c]	73.6% (11.0%)	44.3% (4.4%)	37.9% (7.4%)	43.8% (6.8%)
12% Strain	Work (J), Loaded	4.64×10^{-4} (1.34×10^{-4})	2.33×10^{-3} (1.06×10^{-3})	1.53×10^{-4} (1.05×10^{-4})	2.23×10^{-4} (6.17×10^{-5})
	Work Lost (J), Unloaded	3.48×10^{-4} (7.41×10^{-5})	1.14×10^{-3} (5.25×10^{-4})	6.37×10^{-5} (5.97×10^{-5})	1.05×10^{-4} (4.65×10^{-5})
	% Work Lost [c]	76.9% (9.9%)	48.8% (3.4%)	37.7% (10.8%)	45.9% (8.2%)
16% Strain	Work (J), Loaded	6.62×10^{-4} (2.18×10^{-4})	3.90×10^{-3} (1.69×10^{-3})	2.87×10^{-4} (1.78×10^{-4})	3.55×10^{-4} (6.99×10^{-5})
	Work Lost (J), Unloaded	5.01×10^{-4} (1.14×10^{-4})	1.99×10^{-3} (8.52×10^{-4})	1.12×10^{-4} (8.36×10^{-5})	1.73×10^{-4} (6.17×10^{-5})
	% Work Lost [c]	77.8% (8.9%)	50.9% (3.0%)	36.8% (8.6%)	47.8% (8.0%)

[a] *One standard deviation in parentheses. See text for food processing details.*

[b] *Meat was measured both parallel and perpendicular (perp.) to muscle fiber direction. Each sample was successively loaded-unloaded to 4%, 8%, 12% and 16% strain.*

[c] *Percent work loss was calculated as the difference between the work performed during loading and unloading of the sample (i.e., work lost) divided by the work performed during loading.*

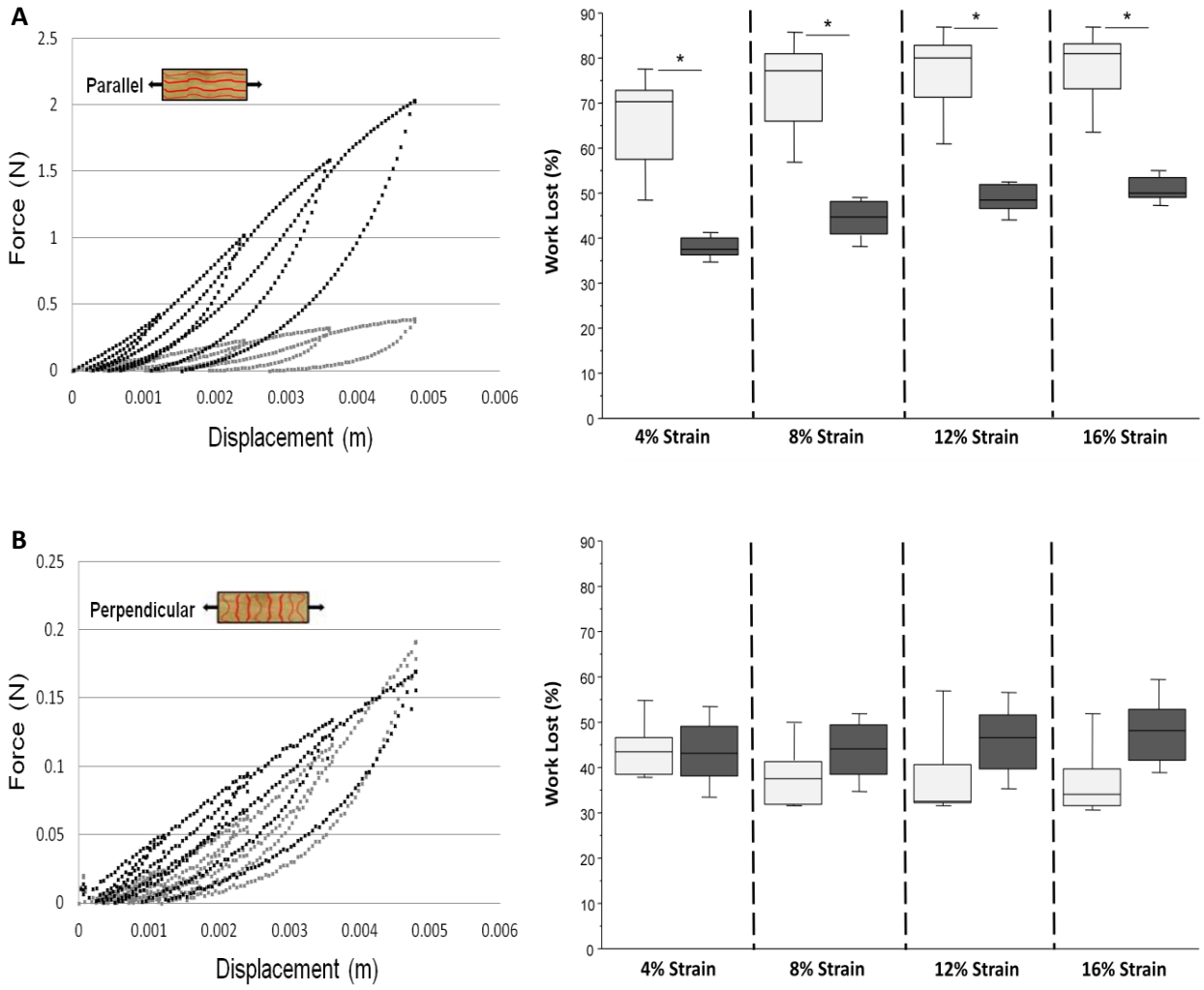

Figure 2.14. *Left: Average force-displacement curves for raw (light) and roasted (dark) meat. Each sample was successively loaded-unloaded to 4%, 8%, 12% and 16% strain. Right: Percent work lost (energy dissipation) during the unloading phase of each work cycle. Meat was loaded in tension both parallel (A) and perpendicular (B) to muscle fiber direction. Box plot whiskers extend to the 10th and 90th percentiles. *p ≤ 0.05, Mann-Whitney U Test.*

DISCUSSION

Tuber material properties responded as predicted to both mechanical tenderization and roasting. Tenderizing reduced tuber toughness by 42%, and roasting reduced toughness slightly more, 49% on average. Roasting the tubers also decreased fracture stress, made them more compliant and increased fracture strain. In contrast, meat did not respond as predicted for all material property measures. Tenderizing the meat by pounding it with a hammerstone did not change toughness, suggesting that this form of processing simply disorganized the muscle fibers and connective tissue, and did not alter their intrinsic properties. Additionally, although roasting significantly increased meat toughness, fracture stress and stiffness, and reduced elastic energy loss, the latter two properties only responded to roasting when measured parallel to fiber direction. Finally, contrary to predictions, roasting meat did not significantly affect the amount of strain necessary for fracture.

What are the implications of this material property data for hominins? The tuber results support the hypothesis that mechanical processing could have played a role in favoring the smaller postcanines and faces of *H. erectus* compared to earlier hominins. Based on fracture scaling, the 42% average toughness decrease measured in tubers pounded with a hammerstone predicts a 24% decrease in postcanine occlusal area, a reduction that coincides with the fossil record (e.g., (Brace, 1967; Wolpoff, 1973; McHenry, 1994; McHenry and Coffing, 2000)). In addition to lower toughness, ingested particle size is also reduced when a tuber is pounded (see Figure 2.2), which may further decrease required masticatory force.

Although the reduction was not as great as the predicted 27%, an additional 17% decrease in tuber toughness occurred with roasting, which could have contributed to the facial and dental size reductions of later *Homo*. As predicted, cooked tuber fracture stress and stiffness was greatly reduced and fracture strain increased. The effects of roasting were largest for yams, possibly due to a higher internal temperature reached during cooking, and also from their higher starch content and the

presence of a unique heat-activated β–amylase that further aids starch gelatinization (Binner et al., 2000).

Agrawal et al. (1998) demonstrated a negative correlation between chewing muscle recruitment and a food's $(\text{toughness}/\text{stiffness})^{0.5}$. To further explore the differential effects of cooking, this index was calculated for the three tubers (stiffness from 20-80% fracture stress was averaged) (Table 2.5). Interestingly, while $(\text{toughness}/\text{stiffness})^{0.5}$ increased ~47% when yams were roasted, it decreased when beets and carrots where cooked. This leads to the prediction that force per chew will actually be higher in roasted versus raw beets and carrots. This force increase may be mitigated, however, by fewer chews overall as increased fragmentation occurs per chew at higher $(\text{toughness}/\text{stiffness})^{0.5}$ (Agrawal et al., 1997). Additionally, cooking may make tubers more nutritionally dense by dehydrating them, increasing digestibility, and lowering cost of digestion (Carmody et al., 2011), all of which might further reduce masticatory effort per calorie of food.

While the tuber data support the idea that mechanical and thermal processing techniques could have allowed for the facial and dental size decreases within *Homo*, the meat results are more difficult to interpret. Contrary to predictions, mechanical tenderization did not affect meat toughness and roasted meat was actually tougher and requires approximately 50 - 220% more stress to fracture than raw meat. These material property changes suggest an increase, not decrease, in hominin dental size and facial robusticity.

Toughness changes, however, do not necessarily mean that more masticatory effort must be expended to chew raw versus roasted meat. As previously discussed, primate molars appear to be poorly adapted to chewing raw meat effectively. Compression does not produce fractures in raw meat; it must be sheared or pulled. (This is why I used cutting and tensile tests to measure meat properties.) For example, the meat used for this study was hit 20 times with a replica Oldowan hammer stone and did not fracture at all, but was instead simply mashed. Cooking may improve masticatory performance

by increasing the efficiency of mastication. First, fractures in meat tend to preferentially run through weaker perimysial bonds as opposed to breaking the tougher muscle fibers (Purslow, 1985; Lewis and Purslow, 1990; Willems and Purslow, 1997; Lillford, 2001). Oral fracture mechanics may therefore be governed more by the material properties of meat that were measured perpendicular, rather than parallel, to fiber direction because connective tissue fractures dominate in the former. Although both fracture stress and toughness increase in this direction with roasting, $(\text{toughness}/\text{stiffness})^{0.5}$ is 23% higher, predicting a decrease in muscle activity per chew (Table 2.5).

Second, roasting stiffens meat parallel to fiber direction and decreases energy loss (hysteresis). Although cracks that run across fibers are rare, comminution efficiency (food breakdown per chew) will increase when they do occur. The maximum number of particles possible from perimysial fracturing is positively correlated with the number of muscle fibers present. In a given volume of food, fiber number is greatest in slices running against the grain of the meat and lowest in cuts following the longitudinal axis of the muscle. In the later case especially, stiffening with roasting may make the food less "displacement limited" and reduce fracture strain. Although ultimate fracture strain was not different between raw and roasted meat, there was a clear dip in stress at approximately 0.3 strain for the roasted meat pulled parallel to fiber direction (Figure 2.12). This dip did not occur in any of the other curves and may indicate fracture initiation, breakage of the first muscle fiber, after which the crack deviates into the weaker perimysium and eventually moves across the remaining fibers (increasing stress) to completely fracture the meat into two pieces. A decrease in energy loss with roasting will further aid fracture propagation and significantly increase the probability of fracture in the limited strain environment between two occluding teeth (Figure 2.14). An approximately 40% decrease in $(\text{toughness}/\text{stiffness})^{0.5}$ strengthens the prediction of more chewing fractures occurring when meat is roasted.

Finally, as with tubers, roasting may make the meat more nutritionally dense, allowing for less consumption to meet the same caloric needs. Water loss was significant with roasting, 35-40% by weight, greatly increasing the amount of nutrients per gram. Digestive costs may also improve with cooking as shown in studies of Burmese pythons (Boback et al., 2007) and mice (Carmody et al., 2011) fed raw versus cooked meat.

Table 2.5. Average toughness and stiffness (elastic modulus) of raw and roasted tubers and meat. [a]

		Toughness *(J/m^2)*	**Stiffness** [b] *(kPa)*	**(Toughness/Stiffness)$^{0.5}$**
TUBERS *(n=10)*	*Raw Beet*	1424.9	4372.7	0.57
	Roasted Beet	622.6	3069.5	0.45
	Raw Carrot	903.8	6007.3	0.39
	Roasted Carrot	553.9	4160.8	0.36
	Raw Yam	774.6	5081.9	0.39
	Roasted Yam	403.0	1227.5	0.57
MEAT [c] [d] *(n=10)*	*Raw Meat (parallel)*	154.3	218.1	0.84
	Roasted Meat (parallel)	218.2	889.6	0.50
	Raw Meat (perpendicular)	277.1	65.9	2.05
	Roasted Meat (perpendicular)	544.6	85.7	2.52

[a] *See text for experimental details.*

[b] *Stiffness (elastic modulus) at 20%, 40%, 60% and 80% fracture stress was averaged.*

[c] *Material properties of meat were measured both parallel and perpendicular to muscle fiber direction.*

[d] *One raw meat sample (measured parallel to muscle fiber direction) did not completely fracture. This sample was omitted from analysis and sample size was reduced to nine.*

Future Research

The data presented in this study highlights the need to test food processing effects on masticatory performance variables. The utility of testing material properties cannot be understated because these tests are relatively inexpensive to perform on a wide variety of foods and processing

types. However, the relationship between food properties and fracture in the oral cavity is complex. For example, the low toughness and high (toughness/stiffness)$^{0.5}$ of raw meat compared to both raw and roasted tubers leads to the prediction of less force per chew when consuming meat. Therefore, it is reasonable to hypothesize that simply adding more raw meat to hominin diets can easily explain smaller, less robust masticatory morphology. A caveat to the material property data, however, is that specific testing environments were used that are conducive to fracture in meat (cutting and tensing). As discussed above, the ability of hominins to actually efficiently comminute this food source raw is questionable; similar to when chimpanzees chew raw meat, significant chewing time and effort may be required and although force per chew may be low when consuming raw meat, the additive effect of a large number of chews may substantially increase total masticatory force. Experimentally testing masticatory performance by measuring chewing forces and the degree of food fracture in the resulting bolus is the only way to conclusively determine if hominins can effectively comminute raw meat. To this end, the following chapter (Chapter 3) details a series of masticatory experiments that address the question of how well, and with what force, humans chew raw and processed foods (including meat).

Future research should also focus on testing the material properties of other food and processing types relevant to early hominins. Pounding and dry roasting are only two of many processing types that would have been available to early hominins, and roasting was performed to a single, relatively high, temperature. Soaking, leaching, chemical tenderization and especially slicing, grinding, and slow drying over a small fire or in the hot sun may have been employed as well. It is also important to note that these methods are not mutually exclusive and multiple processing steps may have been performed on single piece of food. Additionally, food choice will undoubtedly change the effects that processing has on material properties. For example, although boiling greatly decreases potato and white onion toughness, it has no effect on the toughness of white turnips (Lucas, 2004). A number of factors such as the amount of starch and fiber in plants, and age, fat reserves, amount and distribution of

connective tissue in muscle tissue will alter fracture properties. Only four domesticated foods (three tubers and goat meat) were tested and although they are representative of food sources potentially important to early hominins, testing of additional foods, especially wild vegetation and game meat will further inform hominin dietary hypotheses.

CHAPTER 3. MASTICATORY PERFORMANCE

Dear Readers,

While Chapter 2 was written as a single stand-alone publication, Chapter 3 will eventually be split into at least two separate publications, one on the masticatory effects of processing tubers and another on the effects of processing meat. An additional publication may result from the hominin diet models presented in the discussion section. This chapter comprises three separate experiments and the methods and results sections are particularly lengthy. For clarity, each study's methods and results are presented together (as opposed to describing all of the methods, followed by all of the results).

INTRODUCTION

Masticatory effort differs greatly between chimpanzees and modern humans. Chimpanzees can generate an estimated maximum molar bite force of 1500-1750N (Wroe et al., 2010; Constantino et al., 2012) and they spend just under 40% of their day chewing fruits, seeds, and tough leaves and pith (Organ et al., 2011). In contrast, modern human maximum molar bite force is approximately 25-70% lower than chimpanzees' (most measurements range from 400-1300N (Hagberg, 1987; Braun et al., 1995; Hansdottir and Bakke, 2004; Regalo et al., 2008)) and they spend less than 5% of their day consuming a relatively soft, processed diet (Organ et al., 2011). The extreme masticatory differences of modern humans compared to chimpanzees have undoubtedly influenced human cranio-dental morphology.

Masticatory forces affect the skulls and teeth of animals in a number of ways. First, high chewing forces are generated by chewing muscles that have a large physiological cross-sectional area, which is increased either by adding muscle cross-sectional volume or by increasing pennation so that more fibers are packed into a given area. A number of studies have calculated the maximum bite force capabilities of primate and hominin species by estimating muscle size from features on the skull and mandible (e.g., (Demes and Creel, 1988; O'Connor et al., 2005; Eng et al., 2013 (in review))). These

studies have shown that large masticatory muscles (and therefore higher maximum bite forces) tend to correspond with large temporal fossae, as well as increased surface area for attachment of relatively large muscles, which are associated with temporonuchal and sagittal crests (especially in relatively small-brained taxa), and relatively large zygomatic arches and mandibular rami.

Masticatory forces also have more general effects on skull size and shape. Chewing produces a complex pattern of twisting, bending, shearing and wishbone strains in the face and jaws (Hylander, 1984; Hylander et al., 1991; Hylander and Johnson, 1994; Ross and Hylander, 1996; Hylander and Johnson, 1997; Daegling and Hylander, 1998; Ross, 2001; Lieberman et al., 2004), which are mitigated by adding bone in the plane of deformation so that stress (force/area) remains low. Therefore, taller, more orthognathic faces are better able to resist strains in the coronal and sagittal planes, respectively. Additionally, because the highest strains tend to be localized in the occlusal plane and anterior zygomatic arch (Hylander et al., 1991; Lieberman, 1996; Hylander and Johnson, 1997; Lieberman et al., 2004), animals that produce larger chewing forces often have relatively large, robust corpus, palate and zygomatic bones.

It should be noted, however, that regardless of size of the masticatory muscle size and bone robusticity, certain facial shapes are better able to efficiently generate force and resist the resulting strains. The face and jaws can be modeled as a lever system with the jaw joint (glenoid fossa) as the center of rotation (e.g. (Demes and Creel, 1988; O'Connor et al., 2005; Eng et al., 2013 (in review))). The effective mechanical advantage is the ratio between the masticatory muscle in-lever and the bite out-lever, with higher values corresponding to more efficient bite force generation (higher muscle torque relative to bite (resistance) torque). Higher effective mechanical advantage is achieved by flaring the zygomatic arches laterally and increasing the anterior distance between the zygomatic arch and angle of the mandible (both of which increase masseter lever arm length), as well as moving the location of the bite closer to the glenoid fossa by reducing prognathism.

Finally, masticatory force does not just affect the bones of the skull, face and jaws, but also influences the morphology of the dentition in complex ways. Larger postcanine occlusal areas are associated with animals that generate higher bite forces (e.g. (Demes and Creel, 1988; Constantino et al., 2012)). A primary explanation for this correlation is allometry. Larger animals have both larger masticatory muscles (which generate higher forces) and larger postcanine teeth (Gingerich et al., 1982; Fortelius, 1985; Lucas, 2004). Given a certain body size, however, it is reasonable to predict that species consuming foods requiring higher chewing forces will also have relatively larger occlusal areas so that occlusal stress (masticatory force per area) remains relatively constant (Demes and Creel, 1988; Eng et al., 2013 (in review)). Additionally, larger occlusal surface areas can better comminute a wide range of food sizes and also increase the rate of food breakdown by providing more contact points between the food bolus and tooth (Owens et al., 2002; Lucas, 2004). Enamel thickness is also thought to be related to diet, although this relationship is less clear. Thin enamel is typically associated with animals that consume tough foods that must be sheared for efficient comminution, such as leaves, grasses and meat. In contrast, animals that eat harder and/or abrasive foods, especially those containing exogenous grit, tend to have thicker enamel, which is thought to decrease the probability of complete dental fracture and to increase the amount of material that can be worn away without exposing the internal dentine (Lucas et al., 2008; Constantino et al., 2009; Rabenold and Pearson, 2011; Lucas et al., 2013).

Examination of the hominin fossil record reveals evidence of reduced masticatory effort approximately 2.3 mya (Kimbel et al., 1997) with the evolution of the genus *Homo.* Although *H. habilis sensu lato* is represented by only a small number of fossils, from what can be ascertained, they likely had australopith-like postcrania, but derived heads and teeth; compared to gracile australopiths, *H. habilis* appear to have had relatively larger brains and 4-8% smaller postcanine teeth situated in slightly more gracile jaws (e.g., (McHenry and Coffing, 2000; Lieberman, 2011; Wood and Baker, 2011)). In contrast, *H. erectus sensu lato* marks a pronounced transition from earlier hominins (for review, see (McHenry and

Coffing, 2000; Anton, 2003; Klein, 2009; Lieberman, 2011) (N.B., I include in *H. erectus* early east African fossils sometimes attributed to *H. ergaster* and material from Dmanisi, sometime attributed to *H. georgicus*.) *H. erectus* is the oldest hominin to be found out of Africa and representative fossils span a large temporal (approximately 1.9 mya to less than 0.1 mya) and geographical range (from Africa to Asia). Although there is much variation, *H. erectus* generally had a more modern postcranium, with larger body mass, relatively longer legs, relatively shorter arms, and a narrow pelvis and barrel-shaped ribcage, which are indicative of a relatively small gut. Additionally, *H. erectus*, especially later forms, tended to have long, low cranial vaults that housed larger brains than earlier hominin species, and also possessed more vertical faces with smaller masticatory muscles, slightly more gracile masticatory features (i.e., less robust mandibles and zygomatic arches), and postcanine teeth approximately 21-25% smaller than those of *H. habilis* and gracile australopiths, respectively (Brace, 1967; Wolpoff, 1973; Chamberlain and Wood, 1985; McHenry, 1994; McHenry and Coffing, 2000; Lieberman, 2011; Eng et al., 2013 (in review)).

Reduced masticatory robusticity, postcanine size and intestine size (starting with *H. erectus*), coupled with the increased energetic demands of having larger brains and bodies, suggest members of the genus *Homo* consumed higher quality foods that were easier to chew and digest, and/or packed more nutrients per food volume. Dental microwear and carbon isotopic analyses indicate that they had a broad diet composed of C_3 and C_4 foods that, on average, were not particularly hard or tough (Ungar et al., 2006b; Pontzer et al., 2011; Ungar and Sponheimer, 2011; Ungar et al., 2012). What dietary shifts could have lead to the morphological changes seen in the evolution of the genus *Homo*? Two of the most discussed possibilities include the addition of more meat to the diet (e.g., (Hill, 1982; Shipman, 1986; Milton, 1999; Stanford and Bunn, 2001; Bunn, 2007)) and/or the adoption of food processing techniques (e.g., (Wrangham et al., 1999; Lucas, 2004; Wrangham, 2009; Lieberman, 2011)).

Meat is a high quality food that is readily digestible and provides important sources of calories, protein and fat. It is very likely that early *Homo* was consuming more meat than earlier hominins. First, abundant stone tool remains and cut marks on bones indicate increased meat consumption by Oldowan hominins (Bunn, 1981; Bunn and Kroll, 1986; Bunn, 1994; Dominguez-Rodrigo et al., 2002; Plummer, 2004; Dominguez-Rodrigo and Barba, 2006; Bunn, 2007). Additionally, the ratios of Sr/Ca and Ba/Ca in early *Homo* dental enamel is similar to those of carnivores (Balter et al., 2012). Finally, early *Homo*, including *H. habilis*, possessed more occlusal relief than australopiths, which may have aided in the consumption of tough foods such as meat (Ungar, 2004). One problem with meat eating, however, is that it is a highly elastic food that must be sheared between the teeth for efficient comminution (intra-oral food breakdown). The relatively low-cusped, bunodont molars of apes and hominins are poorly adapted to chewing such extensible and fracture resistant foods; chimpanzees have been observed to spend upwards of 4-11.5 hours chewing small (~4 kg) animal carcasses (Goodall, 1986; Wrangham and Conklin-Brittain, 2003). This suggests that hominins would have had to expend a great deal of masticatory effort to consume meat, which does not correspond with the morphological changes evident in the fossil record. To date, however, the ability of humans to effectively chew raw meat has not been quantified.

Another dietary shift that occurred during the evolution of the genus *Homo* is the increased reliance on food processing techniques. Without exception, every modern human population processes their food in some way, including soaking, leaching, chopping, blending, frying, roasting and baking. (Wrangham and Conklin-Brittain, 2003; Wrangham, 2007). Mechanical food processing methods are not unique to humans. Chimpanzees and other animals, such as otters, are known to use rocks to pound open hard objects like nuts and mollusks (Hall and Schaller, 1964; Goodall, 1986; Boesch and Boesch-Achermann, 2000) and it is reasonable to predict that these forms of rudimentary processing were also practiced by the last common ancestor of chimpanzees and hominins. By the time early *Homo* evolved,

however, food processing was likely much more advanced. The oldest stone tools in the archaeological record currently date to 2.6 mya (Semaw et al., 1997), and the earliest evidence of tool-assisted butchery may date to 3.4 mya (McPherron et al., 2010). Analyses of tools and animal remains in the archaeological record indicate that Oldowan hominins extensively used tools to process meat and possibly plant material (Keeley and Toth, 1981; Semaw et al., 2003; Dominguez-Rodrigo et al., 2005; Bunn, 2007; Pobiner et al., 2008). Although their exact uses are unknown, sharp edges on Oldowan choppers and scrappers could have been used to cut and slice, while more blunt edges, such as those found on spheroids and hammerstones could have been used to pound and grind foods.

Another form of food processing that became important at some time during the evolution of the genus *Homo* is cooking. Cooking has a number of benefits that potentially reduce the cost of digestion and increase net energy gain: it detoxifies, reduces endemic parasites (especially important for meat), breaks down proteins and starch, and in many cases makes foods softer and increases digestibility (Stahl, 1984; Wrangham et al., 1999; Wrangham and Conklin-Brittain, 2003; Boback et al., 2007; Carmody and Wrangham, 2009; Wrangham, 2009; Carmody et al., 2011). The benefits of cooking have led Wrangham et al. (1999) to suggest that cooking helped make possible the increased brain and body size, and smaller guts, teeth and less robust faces of *H. erectus,* and possibly even led to reduced sexual dimorphism, central place foraging, mate provisioning and the development of pair-bonds (see also (Wrangham and Conklin-Brittain, 2003; Wrangham, 2009)). When cooking evolved, however, is controversial. The oldest clear evidence for fire in the archaeological record is from burned bones and ashes in Wonderwerk Cave, South Africa, which may push the emergence date to 1 mya (Berna et al., 2012). This evidence, as well as other traces of fire from Gesher Benot Ya'aqov (Goren-Inbar et al., 2004) dated to 790 kya are not necessarily evidence for cooking, and most Middle Pleistocene sites have yielded no evidence of cooking, despite efforts to find them (e.g. Zhoukoudian (Weiner et al., 1998)). Consequently, many researchers believe that habitual cooking is a relatively recent behavior (e.g.,

(Brace, 1995; Ragir, 2000; Bunn, 2007)) because hearths and other features indicative of habitual cooking do not become common until the Middle Paleolithic (see James (1989) for review).

For all of the discussion on dietary transitions relevant to the evolution of the genus *Homo,* the effects of increased meat eating and/or the adoption of food processing techniques on hominin masticatory performance has not been studied. Compared to *H. erectus*, *H. sapiens* have larger brains, and smaller, less robust faces with postcanine teeth that are approximately 15% smaller ((Brace, 1967; Wolpoff, 1973; McHenry, 1994; Lieberman, 2011). A further reduction in masticatory effort is evident when one compares modern *H. sapiens* to older Pleistocene *H. sapiens*, with the former having even smaller, more gracile faces and smaller postcanines (Brace and Mahler, 1971; Brace, 1991; Lahr, 1996; Lieberman, 2011). Do these changes within *H. sapiens* correspond to the advent, and then increasing reliance of cooking techniques, respectively? If so, then are the morphological changes evident in early *Homo*, in particular *H. erectus,* caused by more meat in the diet and/or increased use of mechanical processing techniques? These questions highlight the need for masticatory performance experiments. Without quantifying the effects of food processing on mastication it is difficult to predict their consequences for hominin cranio-dental morphology. Therefore, the goal of this study is to experimentally quantify human masticatory performance resulting from the consumption of foods that are mechanically or thermally processed.

General Hypotheses

The general hypothesis to be tested is that food processing techniques reduce masticatory effort by decreasing chew force and aiding intra-oral food fragmentation. The effect of processing on meat mastication is predicted to be especially pronounced because, as discussed previously, human bunodont postcanines are poorly adapted to shear tough, elastic foods like raw meat. Additionally, it is hypothesized that thermal processing techniques (i.e. cooking) will reduce masticatory force more than

mechanical processing methods. If this is true, then support is gained for the idea of a two-phase shift in hominin food processing, mechanical followed by thermal processing techniques, with the latter being responsible for continued reductions in the size and robustness of the masticatory apparatus in the genus *Homo*. If thermal processing methods do not further reduce masticatory force relative to mechanical techniques, then other dietary changes, such as increased consumption of calorically-dense meat, must have been played a key role in reducing masticatory effort.

Foods and Processing Techniques

Before outlining specific predictions concerning the effects that particular processing methods will have on mastication, it is first necessary to discuss the foods and types of processing that will be studied. In order to allow for direct comparison of material property and masticatory performance changes resulting from processing, the same foods (goat meat and three tubers: beets, carrots and yams) and processing techniques (mechanical tenderization and dry roasting) that were used in Chapter 2 are also used in this study. An additional mechanical processing technique, slicing, is also tested. Although slicing does not change a food's intrinsic material properties, it does reduce ingested particle size and, like mechanical tenderization (pounding), could be performed by any hominin with an Oldowan toolkit.

As discussed in the previous chapter, there are a number of reasons to focus on both meat and tubers, a type of underground storage organ. First, underground storage organs are thought to have been an especially important food source for early hominins (e.g., (Hatley and Kappelman, 1980; Laden and Wrangham, 2005; Ungar et al., 2006a) and thus provide a good comparison to meat. Additionally, tubers and meat are very different from a material property (see Chapter 2), and therefore masticatory perspective. Meat is composed of elastic fibers hierarchically bound with connective tissue and is a tough, yet compliant food. In contrast, raw tubers comprise a latticework of fluid-filled cells under

significant turgor pressure. Although tubers, especially some non-domesticated, wild varieties, are extremely tough (Dominy et al., 2008) and probably require high masticatory forces to consume, compared to raw meat, fractures readily occur when they are compressed between primate/hominin teeth. Therefore, hominins consuming these foods face two different masticatory challenges, high total chew force for meat versus high forces per bite for tubers, and it is reasonable to predict that processing will have different effects on the mastication of these foods.

Specific Hypotheses (Table 3.1)

Agrawal and colleagues have shown a strong relationship between the material properties of a wide range of food types (from nuts to soft cheeses) and masticatory muscle recruitment and fracture rates (Agrawal et al., 1997; Agrawal et al., 1998). As toughness and stiffness (elastic modulus) or a combination of these two properties, $(\text{toughness} \times \text{stiffness})^{0.5}$ increased, muscle recruitment used for the first bite and the resulting amount of fragmentation also increased. In contrast, another combination of toughness and stiffness, $(\text{toughness}/\text{stiffness})^{0.5}$, was negatively correlated with muscle recruitment and food breakdown rates. Based on these data, tougher and stiffer foods should require more masticatory force per chew, yet because the food is fractured more readily in the oral cavity, fewer chews will be used and total masticatory force (number of chews X force per chew) may be lower. This prediction leads to the following specific hypotheses regarding the effects of food processing techniques on four masticatory performance variables, number of chews, force per chew, total masticatory force and comminution efficiency (the degree of intra-oral food fragmentation).

Slicing

Slicing does not affect tuber or meat intrinsic material properties, with the exception of fracture stress and strain, which are higher in smaller structures. The smaller size of ingested particles, however,

means that overall force per chew will likely be reduced (Kohyama et al., 2004b; Xu et al., 2008). Additionally, the pre-fractured state of the food prior to consumption will decrease both the number of chews and the total force required for mastication, as well as increase comminution efficiency (amount of food fragmentation per chew).

Mechanical Tenderization

As shown in Chapter 2, mechanical tenderization by pounding with an Oldowan hammerstone fractures and damages tuber internal cellular structure, greatly decreasing measured toughness. In contrast, raw meat is extremely difficult to break into multiple pieces without a sharp cutting edge. Thus, unlike its effect on tubers, pounding with an Oldowan hammerstone does not fracture meat into multiple pieces, but instead acts to primarily disorganize the uniform arrangement of the muscle fiber bundles (see Chapter 2, Figure 2.2), which has no effect on measured toughness. These opposing effects of mechanical tenderization on tubers versus meat will lead to different masticatory responses.

Tubers: Mechanically tenderizing tubers decreases their toughness and this will lead to a reduction in force used per chew during mastication. Additionally, chew number and total masticatory force will be lower, and comminution efficiency higher compared to raw tubers, because the food is broken into multiple, smaller fragments prior to ingestion. These masticatory performance changes will be even greater than those resulting from slicing because in addition to pre-oral fracturing, the food's material properties are also altered with mechanical tenderization.

Meat: Toughness of meat does not change with pounding and therefore the amount of masticatory force that a person uses per chew when consuming mechanically tenderized meat will similarly remain unchanged. Additionally, because the meat is not fractured into multiple pieces prior to consumption, chew number, total masticatory force and particle size at swallow will remain unaffected by tenderizing.

Roasting

Roasting tubers and meat will reduce masticatory effort, but this is achieved through two different mechanisms.

Tubers: Roasting decreases tuber toughness, stiffness and fracture stress and because of these material property changes, force per chew will be lower for roasted versus raw tubers. On average, however, total number of chews may increase because less tough and stiff foods do not breakdown as readily. Even if roasted tubers are chewed more times, force reductions per chew are predicted to be large enough to decrease total masticatory force relative to raw and mechanically processed tubers.

It is possible that there will be some variation in tuber-specific response to roasting. In Chapter 2 it was shown that $(\text{toughness}/\text{stiffness})^{0.5}$ decreased when beets and carrots were roasted, but increased when yams were roasted (Table 3.2). This means that roasting beets and carrots may increase force per chew, but decrease chew number and total masticatory force, while the opposite is true for yams (roasting decreases force per chew, but increases chew number and total masticatory force). Compared to other foods such as soft mozzarella cheese (0.73) and brittle Brazil nuts (0.07), however, the measured difference in $(\text{toughness}/\text{stiffness})^{0.5}$ among raw and roasted tubers is very low (0.39 - 0.57). (Mozzarella and Brazil nut ratios were calculated from Agrawal et al. (1997). Stiffness was changed from MPa to kPa in order to make the ratios comparable.) The small $(\text{toughness}/\text{stiffness})^{0.5}$ differences amongst the tubers will likely dampen the predictive power of this index. Additionally, tubers are a 'force-limited' food that requires high force relative to strain in order to fracture. In these types of foods, masticatory performance may be better explained by $(\text{toughness} \times \text{stiffness})^{0.5}$ (Lucas, 2004) and because this decreased for all of the roasted tubers, the direction of the masticatory response to roasting should be the same for carrots, beets and yams.

Meat: Unlike tubers, the toughness and fracture stress of meat is significantly increased when it is roasted, which means that force per chew will also be higher. Meat is a 'space-limited' food that

requires relatively high strains to fracture, however, and the ratio between toughness and stiffness is particularly informative for masticatory performance. Roasting makes meat more brittle, reducing $(\text{toughness/stiffness})^{0.5}$ and although roasted meat may require more force to per chew than raw or tenderized meat, total masticatory force will be lower as fewer chews are required to reach the same degree of comminution (intra-oral food fragmentation). Improved comminution, which is further aided by less elastic energy loss with roasting, may prove to be especially significant because breakdown of raw and tenderized meat in the oral cavity is predicted to be extremely difficult to accomplish.

Table 3.1. Masticatory performance predictions.

		Lowest → ***Highest***
TUBERS	Number of Chews	*Tenderized* -·-·-> *Sliced* -·-·-> *Raw* -·-·-> *Roasted*
	Force per Chew	*Roasted* -·-·-> *Tenderized* -·-·-> *Sliced* -·-·-> *Raw*
	Total Force	*Roasted* -·-·-> *Tenderized* -·-·-> *Sliced* -·-·-> *Raw*
	Comminution Efficiency	*Roasted* -·-·-> *Raw* -·-·-> *Sliced* -·-·-> *Tenderized*
Meat	Number of Chews	*Roasted* -·-·-> *Sliced* -·-·-> *Tenderized* = *Raw*
	Force per Chew	*Sliced* -·-·-> *Tenderized* = *Raw* -·-·-> *Roasted*
	Total Force	*Roasted* -·-·-> *Sliced* -·-·-> *Tenderized* = *Raw*
	Comminution Efficiency	*Tenderized* = *Raw* -·-·-> *Sliced* -·-·-> *Roasted*

Table 3.2. Material properties of raw and roasted tubers and meat. [a]

	Toughness *(J/m^2)*	**Stiffness** [b] *(kPa)*	**(Toughness x Stiffness)$^{0.5}$**	**(Toughness/Stiffness)$^{0.5}$**
Raw Beet (n=10)	1424.9	4372.7	2496.2	0.57
Roasted Beet (n=10)	622.6	3069.5	1382.4	0.45
Raw Carrot (n=10)	903.8	6007.3	2330.1	0.39
Roasted Carrot (n=10)	553.9	4160.8	1516.9	0.36
Raw Yam (n=10)	774.6	5081.9	1984.1	0.39
Roasted Yam (n=10)	403.0	1227.5	703.4	0.57
Average Raw Tuber (n=30)	1057.4	5154.0	2334.4	0.45
Average Roasted Tuber (n=30)	526.2	2819.3	1218.0	0.43
Raw Meat [c] *(n=10)* [d]	215.7	138.0	172.5	1.25
Roasted Meat [c] *(n=10)*	381.4	487.7	431.3	0.88

[a] *See Chapter 2 for experimental details.*

[b] *Stiffness (elastic modulus) at 20%, 40%, 60% and 80% fracture stress was averaged.*

[c] *Meat material properties were measured both parallel and perpendicular to muscle fiber direction and were averaged.*

[d] *One raw meat sample (measured parallel to muscle fiber direction) did not completely fracture. This sample was omitted from analysis and sample size was reduced to nine.*

METHODS

Sample Preparation - TUBERS.

Organic tubers, jewel yams (*I. batatas*), carrots (*D. carota*) and red beets (*B. vulgaris*), were purchased from a local grocery store. Each tuber was divided into 2 portions. One portion was cut into 17 mm thick transverse slices for roasting (below) and the other was cut into 13 X 13 X 13 mm cubes, avoiding the outer tuber cortex (some carrot samples contained a very small portion of the cortex). Sample dimensions were measured using digital calipers (accuracy, ± 0.01mm) and weighed on a digital scale (accuracy, ±0.1g). Cube weight did not differ among the three tubers (Yam 2.2±0.05 g; Carrot 2.3±0.05 g; Beet 2.2±0.05 g).

Cubes were left unprocessed (raw samples), or were processed by either slicing them into eight smaller cubes with the approximate dimensions of 6.5 X 6.5 X 6.5 mm (sliced samples), or by hitting them 6 times with a replica Oldowan hammerstone (tenderized samples). Pounding with a hammerstone tended to break the sample into relatively large and intact pieces (see Chapter 2, Figure 2.2). Roasted samples were created following the roasting protocol described in Chapter 2. Briefly, the 17 mm slices were cooked for 15 minutes on a warmed-up, tabletop propane grill (Perfect Flame™) with the lid open and the gas flow set to "high". To adjust for differences in grill surface temperature, slices were rotated to a different spot on the grill every 2.5 minutes. They were also turned over halfway through the cooking processes to ensure even heating. Roasting in this manner cooked yams to an internal temperature of 89.0±2.7°C, carrots to 78.5±1.1°C, and beets to 78.6±2.2°C (see Chapter 2, Table 2.1). After roasting, the samples were transferred to a refrigerator (4°C) to halt the cooking process. Once cooled to room temperature, the outer cortex and the charred surface in contact with the grill surface were removed. 13 X 13 X 13 mm cubes were then cut from the remaining portion of the cooked

slice. Roasted yam, carrot and beet samples weighed the same (Yam 2.6±0.05 g; Carrot 2.6±0.05 g; Beet 2.6±0.05 g) and were approximately 16% heavier (i.e. denser) than their raw counterparts. After preparation, samples were stored in plastic Ziploc bags or sealed plastic vials at 4°C until immediately prior to the start of the experiment. All samples were used within 12 hours of processing.

Sample Preparation - MEAT.

An adult female goat (*C. aegagrus*) was purchased from a local farm and slaughterhouse (Blood Farms, Groton, MA) and the fresh carcass transported on ice to the Skeletal Biology Lab, Harvard University. Muscle groups were removed using aseptic procedures, sealed in vacuum bags and stored at -20°C. Muscles from the neck and epaxial regions were defrosted at 4°C for approximately 12-24 hours prior to data collection. Samples were randomized to include meat from both neck and epaxial muscles.

3.0 gram samples of meat were cut from defrosted muscles (digital scale, accuracy = 0.1g). These samples were either left unprocessed (raw samples) or were cut into eight, approximately equal sized pieces (sliced samples). Tenderized samples were created by cutting the muscle into a 50.0 gram steak and pounding it 50 times with a replica Oldowan hammerstone. Processing in this manner disorganized the muscle fibers, resulting in a 'mashed' appearance, but did not fracture the steak into separate pieces (see Chapter 2, Figure 2.2). After tenderizing, 3.0 gram samples were cut from the pounded steaks. Roasted samples were created by cooking steaks on a pre-heated tabletop propane grill (Perfect Flame™) with the lid open and the gas flow valve set to "high". Internal temperature was monitored using a digital thermocouple and needle probe inserted into the steak center (Thermoworks™, accuracy ± 0.1°C). Steaks were flipped regularly to ensure even heating and were roasted to a final internal temperature equal to medium-rare (warm, red center, ~55°C) or medium-well (slight pink center, ~ 70°C). On average, roasting goat steaks to medium-rare required 16.3±2.5 minutes of cooking time and reduced water content (weight) by 18.9±2.3% (Table 3.3) Roasting to medium-well

increased both of these values; average cook time was 25.0±5.3 minutes and water (weight) loss was 26.8±5.6%. After roasting , 3.0 gram samples were cut from the steaks, avoiding the charred outer surfaces. All processed samples were stored in sealed plastic containers at 4°C and were used within 12 hours.

Table 3.3. Weight change (water loss), cook time, and internal temperature of three goat steaks roasted to medium-rare or medium-well on a tabletop propane grill.

	Pre-Roasted Weight *(g)*	Post-Roasted Weight *(g)*	% Reduction		Cook Time *(min)*	Internal Temperature [a] *(°C)*	Internal Appearance
Medium-Rare *(n=3)*	227.7	190.2	16.5		19	54	*Warm, red center*
	150.7	118.9	21.1		14	54	*Warm, red center*
	320.5	258.9	19.2		16	55	*Warm, red center*
		Average (S.D.)	***18.9% (2.3%)***		***16.3 min (2.5 min)***	***54.3°C (0.6°C)***	
Medium-Well *(n=3)*	272.9	204.2	25.2		23	69	*Slight pink center*
	212.4	142.4	33.0		31	68	*Slight pink center*
	242.7	189.0	22.1		21	70	*Slight pink center*
		Average (S.D.)	***26.8% (5.6%)***		***25.0 min (5.3 min)***	***69.0°C (1.0°C)***	

[a] *Temperature was measured with a needle-probe thermocouple inserted into the center of the steak.*

Experimental Protocol

Three experiments were performed to analyze the masticatory consequences of food processing. Tubers were tested in the first two experiments (experiment #1 - masticatory force; experiment #2 - comminution performance) and meat was tested in the third experiment (masticatory force and comminution performance combined into a single data collection session). The three experiments and their associated analyses are described below. For clarity, the methods and results of each individual experiment are described together. Even though there is some repetition between protocols, the methods for each experiment are described in full.

(Note: The results of these masticatory experiments were not Bonferroni corrected. Bonferroni is a conservative test employed to reduce type I error (i.e., "false positives"), but unfortunately does so at the risk of increasing type II errors (i.e., "false negatives"). The data from these experiments are already prone to type II errors because the effect size is low (Nakagawa, 2004); regardless of the magnitude of masticatory changes that result from food processing, the standard error is predicted to be high because of the relatively small samples sizes available for performance experiments (8-14 subjects participated in the three experiments outlined below), as well as an expected high standard deviation in the data as masticatory performance is subject to substantial inter-individual variability. Therefore, in order to avoid compounding the risk of type II errors, significance was set to a typical alpha level of $p<0.05$. For comparison, the Bonferroni corrected alpha level for the data would be $p< 0.008$ (tuber experiments) and $p<0.005$ (meat experiments).)

Methods and Results: Experiment #1, Chewing Force (TUBERS)

Methods

Data Collection. 14 adult subjects (7M, 7F) participated in the experiment. Subject age averaged 29±8 years and ranged from 22 to 52 years. All subjects had a complete set of permanent teeth with the exception of the 3rd molars, which were present in only two subjects (male); one subject had all four third molars, and one subject was missing the upper right third molar.

Cleartrace™ surface electromyography (EMG) electrodes (Conmed Corporation) were placed over each subject's right (balancing-side) and left (working-side) superficial masseter and anterior temporalis, and a ground placed on the back of the hand. Electrodes were connected to a grounded pre-amplifier linked to a MA300™ EMG amplifier (Motion Lab Systems, Inc.). Analog signals were passed

through a PowerLab™ 16sp A/D board (ADinstruments, Inc.) and the data was captured at 1000 Hz in LabChart v7 (ADinstruments, Inc.).

After EMG electrode placement, the subjects completed an EMG – force calibration trial using a small, dime-sized Kistler SlimLine™ force transducer (output voltage calibrated to known forces, r^2 = 0.99) connected to a Kistler Dual Mode Amplifier™ linked to the EMG A/D board. Output voltage was captured at 1000 HZ in LabChart v7. In order to ensure a comfortable and sterile biting surface, the top and bottom of the transducer was fitted with a thin (2.4 mm) layer of rubber and was then loosely covered with a single layer of water proof tape and a sterile plastic sleeve. The post-wrapped transducer was 8.8 mm tall and had a diameter of 14.1 mm. Tape was wrapped around the plastic sleeve and wire where it projected from the side of the transducer. This created a 1 to 2 inch 'handle' that was used to hold the transducer comfortably in place within the mouth. Before use, the transducer was allowed to sit in the oral cavity for at least 5 minutes to equilibrate to oral temperatures. The experimenter then placed the transducer between the left first molars and instructed the subjects to bite down with sub-maximal force and then release. Muscle EMG activity and the resulting bite forces were recorded. Subjects repeated this bite-and-release procedure approximately 30 times, with the experimenter viewing the output forces in real time and instructing the subjects to bite more or less hard to ensure a wide array of sampled bite forces.

Once the calibration trial was complete, the transducer was removed and the subjects were presented with samples of raw and processed yams, carrots and beets in random order. They were instructed to eat the samples in a normal manner (chew and swallow), with the exception that they should only chew on the left side so that balancing and working sides could be easily identified. Each sample type was repeated 3 times.

Data Analysis. Transducer force data (peak force (N) and integrated force (N*s) per bite) were measured directly in Chart v7 (Figure 3.1). EMG data was processed using custom written matlab codes

following Lieberman et al. (2006). Briefly, signals were filtered using a Butterworth bandpass filter (4th order zero-lag) with 60 and 300 Hz frequency cut offs. The data was then rectified and binned using a 5 millisecond integral reset, and background muscle activity was removed using Thexton's randomization method (Thexton, 1996). Swallows were identified by a non-uniform EMG pattern lacking the firing of all four masticatory muscles and were omitted from the data set. For each chewing bite, maximum amplitude (v) and integrated area (V*s) of the EMG signal was recorded for the four masticatory muscles (Figure 3.1). Total peak voltage and integrated voltage were calculated by summing the voltage parameters of the chews used to eat each sample.

Two sets of analyses were performed. All calculations were performed in Excel (Microsoft 2007) and StatView statistical package (SAS Institute, version 5.0.1).

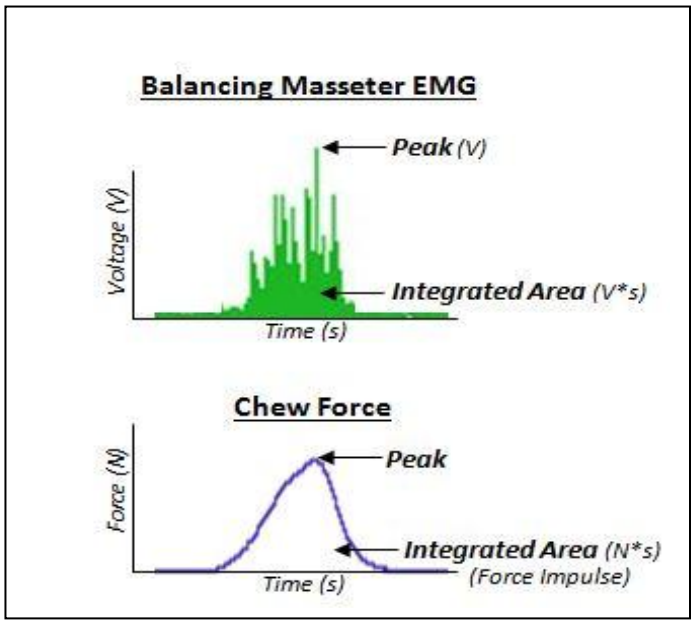

Figure 3.1. *Balancing masseter EMG and the resulting chew force generated during an EMG-force calibration trial. Quantified parameters included peak and integrated EMG voltage and force per chew. Integrated force per chew is particularly relevant to mastication because it is the force impulse, which is a measure of the amount of energy that can be used for food fracture.*

Analysis #1 (EMG Activity). Each subject's muscle EMG parameters (peak and integrated voltage per chew and total per sample) resulting from consuming processed tuber samples were normalized to the corresponding raw sample parameters and the percent change calculated. Within-subject sample triplicates were averaged and the yam, carrot and beet data were analyzed both separately and pooled together to measure average response to tuber processing. A non-parametric one-sample sign test was used to determine whether the sliced, tenderized or roasted values were significantly larger (positive number) or smaller (negative number) than zero, indicating increased or decreased muscle recruitment when consuming processed foods, respectively. Significance was set to $p \leq 0.05$. Each muscle was analyzed separately and pooled together to assess average muscle response to chewing processed tubers.

- percent change = 100*((processed food parameter – raw food parameter) / raw food parameter)

Analysis #2 (Masticatory Force). The second analysis quantified the effects of food processing on chewing forces. Although the EMG data allows for consideration of muscle-specific changes resulting from processing, comparisons of masticatory force better inform overall masticatory morphology.

For each subject, the number of chews taken to eat each sample was noted. Chew force was then calculated from the EMG activity of the balancing-side masseter using subject-specific EMG-force calibration equations created from the calibration trials. Specifically, integrated force and peak force created per bite were linear regressed on the integrated EMG and peak voltage of the balancing-side masseter activity, respectively. The resulting regression equations were then used to transform EMG activity from the chewing trials into forces. Only the balancing-side masseter was used because Proeschel and Morneburg (2002) found a different EMG-force relationship between isometric bites, like those used in calibration experiments, and chewing bites for all muscles except the balancing-side

masseter. Although they only analyzed peak force and masticatory EMG voltage, it is reasonable to predict that the relationship between muscle recruitment and force production should be the same for integrated values.

After calibration, both average force per chew and the total force (average force per chew multiplied by number of chews) used to masticate each sample were calculated. Within-subject sample triplicates were averaged. Yam, carrot and beet data were analyzed both separately and pooled together to measure average response to tuber processing. Significant differences ($p \leq 0.05$) among raw and processed tubers were tested using a Wilcoxon signed rank test.

RESULTS

Tubers - EMG Recruitment. (Table 3.4 and Table 3.5)

Roasting significantly reduced all measures of muscle recruitment. On average, peak EMG voltage per chew of roasted tubers was 13.7±6.5% lower than that of raw tubers ($p<0.001$) (Figure 3.2). An even greater reduction, 21.4±10.5% ($p<0.001$), occurred when comparing total peak voltage of roasted versus raw samples. Comparisons of integrated EMG voltage indicate a similar pattern (Figure3.2): roasting reduced integrated voltage per chew 14.1±6.8% ($p<0.01$) and total integrated voltage 22.0±10.5% ($p<0.001$). As with peak voltage, the reduction was highest when total values per sample (peak and integrated EMG voltage) were calculated, which was driven by fewer chews used to masticate the roasted samples (below). When tubers were analyzed separately, yam consumption was the most affected by roasting (35.6% average reduction), followed by carrots (12.3% average reduction). For beets, only average integrated EMG voltage used per chew was significantly reduced with roasting (5.4±6.8%; $p=0.01$).

While roasting caused the largest reduction of masticatory muscle recruitment, tenderizing tubers also had an effect. Compared to raw tubers, tenderized samples required 4.5±6.1% less

integrated EMG voltage per chew to masticate ($p=0.01$). Total integrated voltage and peak voltage (both per chew and total) used to chew tenderized samples did not differ from raw tubers. Analyzing the tubers separately, only beet consumption was significantly affected by tenderizing. Average integrated EMG voltage used, both per chew (6.8±7.6%) and total (9.9±13.3%), was lower for tenderized versus raw beets ($p<0.01$ and $p=0.01$, respectively). Slicing the tubers had no significant effect on masticatory muscle recruitment.

The above comparisons were made between average muscle response to processing (i.e. balancing and working side temporalis and masseter values were averaged together). It is interesting to note, however, that processing the tubers did not always significantly change the activity of each muscle. For example, on average, only the balancing side masseter activity was significantly reduced when tubers were tenderized ($p=0.01$ for all measurements), suggesting a change in masticatory kinematics.

Table 3.4. Average percent change of peak muscle voltage resulting from masticating size-standardized processed tubers, relative to raw samples. [a]

		Peak Voltage per Chew *(v)*					Total Peak Voltage [b] *(v)*				
		Balancing Side		Working Side		***Muscle Average***	Balancing Side		Working Side		***Muscle Average***
		Masseter	*Temporalis*	*Masseter*	*Temporalis*		*Masseter*	*Temporalis*	*Masseter*	*Temporalis*	
Yam	Sliced	2.6 (12.3)	7.5 (9.7)	3.3 (9.3)	6.1 (8.0)	3.8 (8.2)	6.0 (15.0)	10.5 (11.4)	6.3 (12.2)	9.4 (15.2)	6.8 (12.3)
	Tenderized	-4.4 (11.2)	3.7 (9.4)	3.7 (20.3)	4.7 (10.7)	0.6 (9.8)	-13.3 (14.5)	-6.0 (15.1)	-6.4 (17.9)	-5.3 (16.6)	-8.7 (14.7)
	Roasted	-25.2 (13.8)	-33.7 (15.5)	-26.2 (15.3)	-26.7 (15.1)	-27.0 (14.4)	-42.5 (19.5)	-50.7 (18.8)	-43.1 (20.7)	-45.5 (19.4)	-43.8 (19.6)
Carrot	Sliced	0.6 (6.9)	1.2 (9.6)	0.1 (7.6)	4.5 (10.1)	1.7 (7.8)	3.3 (10.8)	2.0 (9.9)	3.4 (13.3)	5.7 (11.5)	4.5 (11.7)
	Tenderized	-6.3 (13.6)	-2.5 (10.3)	0.5 (8.5)	-0.4 (8.2)	-1.6 (7.2)	-7.8 (14.4)	-6.9 (11.6)	0.2 (19.6)	-4.4 (12.7)	-2.5 (14.8)
	Roasted	-9.3 (8.6)	-12.2 (10.9)	-10.3 (8.4)	-6.9 (5.4)	-9.5 (6.2)	-15.2 (9.4)	-19.7 (14.3)	-14.8 (14.2)	-14.0 (12.8)	-14.8 (11.6)
Beet	Sliced	5.6 (9.8)	7.0 (12.0)	9.4 (25.6)	2.8 (9.0)	4.2 (8.9)	13.4 (24.4)	17.2 (30.2)	15.9 (31.9)	11.5 (23.6)	11.6 (23.3)
	Tenderized	-8.6 (11.1)	-2.2 (12.6)	-1.2 (4.4)	-1.1 (5.9)	-2.7 (5.5)	-11.5 (13.6)	-4.7 (19.6)	-4.6 (11.2)	-4.0 (13.4)	-5.8 (12.7)
	Roasted	-1.8 (7.8)	-2.6 (7.9)	-5.5 (13.4)	-3.6 (6.1)	-4.6 (6.9)	-3.2 (14.7)	-4.0 (17.8)	-6.4 (20.0)	-4.9 (17.1)	-5.7 (16.2)
Tuber Average	Sliced	2.9 (6.2)	5.2 (4.1)	4.3 (7.6)	4.5 (2.9)	3.2 (3.5)	7.6 (12.4)	9.9 (13.4)	8.5 (12.3)	8.9 (11.8)	7.6 (11.1)
	Tenderized	-6.4 (7.3)	-0.3 (4.9)	1.0 (6.4)	1.1 (4.0)	-1.3 (3.4)	-10.9 (8.7)	-5.8 (10.1)	-3.6 (7.3)	-4.5 (8.8)	-5.7 (7.6)
	Roasted	-12.1 (7.0)	-16.2 (7.6)	-14.0 (9.6)	-12.4 (6.0)	-13.7 (6.5)	-20.3 (9.0)	-24.8 (11.6)	-21.4 (13.2)	-21.5 (10.7)	-21.4 (10.5)

[a] *See text for experimental details. Percent change = 100*((Peak Voltage $_{Raw\ Food}$ – Peak Voltage $_{Processed\ Food}$) /Peak Voltage $_{Raw\ Food}$)). One standard deviation in parentheses. Significant changes relative to raw samples are shaded in dark grey, $p \leq 0.05$, non-parametric one-sample sign test. N=14.*

[b] *Total peak voltage = average peak voltage per chew X number of chews*

Table 3.5. Average percent change of integrated muscle voltage resulting from masticating size-standardized processed tubers, relative to raw samples. [a]

		Integrated Voltage per Chew *(V*s)*					**Total Integrated Voltage** [b] *(V*s)*				
		Balancing Side		**Working Side**		***Muscle Average***	**Balancing Side**		**Working Side**		***Muscle Average***
		Masseter	*Temporalis*	*Masseter*	*Temporalis*		*Masseter*	*Temporalis*	*Masseter*	*Temporalis*	
Yam	Sliced	0.1 (9.5)	3.6 (8.9)	-0.3 (10.7)	3.4 (9.4)	0.8 (5.6)	3.1 (10.5)	6.6 (11.8)	2.4 (10.0)	6.7 (13.7)	3.8 (9.4)
	Tenderized	-4.9 (10.1)	-2.2 (12.0)	-0.9 (19.8)	0.2 (10.7)	-2.3 (9.9)	-13.1 (15.8)	-11.1 (17.0)	-10.0 (18.3)	-9.0 (17.3)	-10.9 (16.1)
	Roasted	-24.2 (14.8)	-32.1 (13.6)	-25.7 (13.0)	-30.7 (15.4)	-27.3 (14.7)	-42.0 (20.2)	-49.9 (16.8)	-43.6 (19.1)	-48.3 (19.8)	-44.3 (20.1)
Carrot	Sliced	-0.2 (8.9)	-1.4 (10.3)	-0.6 (10.0)	4.0 (14.3)	0.7 (8.8)	2.4 (12.1)	-0.8 (10.5)	2.7 (15.9)	4.7 (13.5)	3.5 (13.2)
	Tenderized	-8.7 (13.3)	-6.1 (10.3)	-3.5 (8.8)	-2.3 (13.1)	-4.4 (8.7)	-9.9 (16.4)	-10.5 (12.4)	-4.1 (17.8)	-6.6 (14.3)	-5.4 (14.9)
	Roasted	-7.3 (7.9)	-9.7 (8.2)	-13.3 (9.9)	-6.2 (9.3)	-9.7 (6.2)	-13.6 (10.1)	-17.3 (14.5)	-17.6 (16.0)	-13.5 (16.9)	-15.0 (13.0)
Beet	Sliced	-3.1 (14.9)	2.4 (7.8)	8.3 (28.8)	-2.8 (8.7)	-0.2 (8.3)	4.1 (27.3)	11.6 (23.9)	14.6 (35.4)	5.8 (23.7)	6.8 (22.8)
	Tenderized	-11.9 (15.5)	-7.0 (10.1)	-4.4 (8.9)	-7.6 (7.2)	-6.8 (7.6)	-14.8 (17.2)	-9.5 (16.1)	-8.1 (13.1)	-9.8 (14.8)	-9.9 (13.3)
	Roasted	-4.1 (8.0)	-2.3 (6.1)	-6.3 (14.8)	-4.8 (7.5)	-5.4 (6.8)	-5.8 (14.0)	-4.0 (13.2)	-7.1 (22.2)	-6.6 (14.8)	-6.7 (14.9)
Tuber Average	Sliced	-1.1 (7.6)	1.5 (4.4)	2.4 (10.2)	1.5 (5.4)	0.5 (3.4)	3.2 (12.8)	5.8 (11.7)	6.6 (14.2)	5.7 (13.3)	4.7 (11.0)
	Tenderized	-8.5 (9.1)	-5.1 (8.0)	-3.0 (7.9)	-3.2 (7.4)	-4.5 (6.1)	-12.6 (12.5)	-10.4 (12.2)	-7.4 (10.0)	-8.5 (11.5)	-8.7 (10.8)
	Roasted	-11.9 (6.9)	-14.7 (6.9)	-15.1 (10.0)	-13.9 (7.6)	-14.1 (6.8)	-20.5 (9.3)	-23.8 (10.0)	-22.8 (13.5)	-22.8 (11.4)	-22.0 (10.5)

[a] *See text for experimental details. Percent change = 100*((Integrated Voltage $_{Raw\ Food}$ – Integrated Voltage $_{Processed\ Food}$) /Integrated Voltage $_{Raw\ Food}$)). One standard deviation in parentheses. Significant changes relative to raw samples are shaded in dark grey, $p \leq 0.05$, non-parametric one-sample sign test. N=14.*

[b] *Total integrated voltage = average integrated voltage per chew X number of chews*

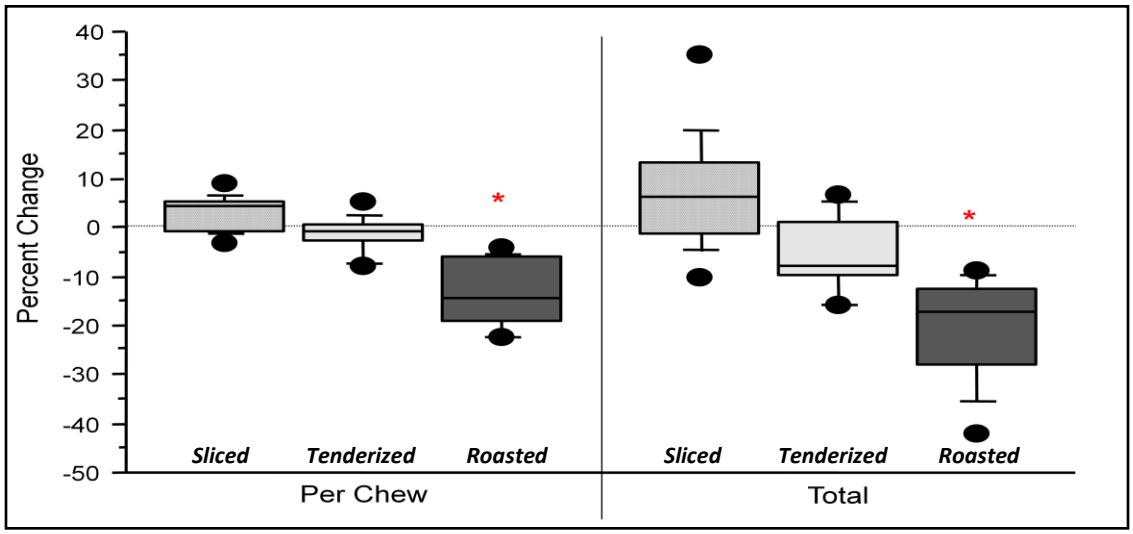

Figure 3.2. *Percent change of peak muscle voltage per chew and total peak muscle voltage (sum of peak voltages per chew) resulting from masticating size-standardized sliced (hatch-mark), tenderized (light grey) and roasted (dark grey) tubers, relative to raw samples. Tubers (yam, carrot and beet) and muscles (working and balancing side masseter and temporalis) averaged. Percent change = 100*((Peak Voltage* $_{Raw\ Food}$ *– Peak Voltage* $_{Processed\ Food}$*) /Peak Voltage* $_{Raw\ Food}$*)). Box plot whiskers extend to the* 10^{th} *and* 90^{th} *percentiles.*p ≤ 0.05, non-parametric one-sample sign test.*

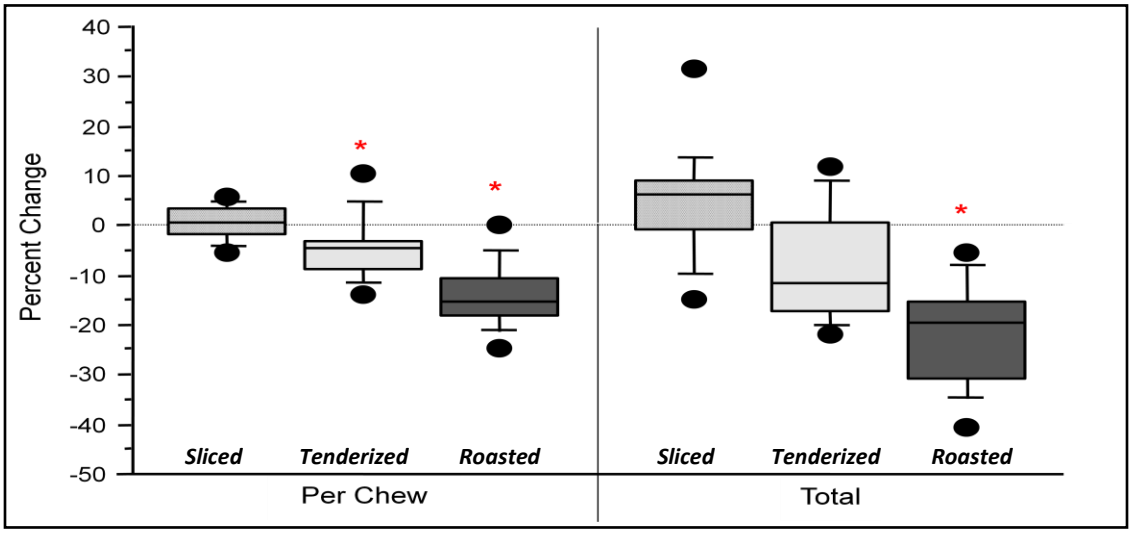

Figure 3.3. *Percent change of integrated muscle voltage per chew and total integrated muscle voltage (sum of integrated voltages per chew) resulting from masticating size-standardized sliced (hatch-mark), tenderized (light grey) and roasted (dark grey) tubers, relative to raw samples. Tubers (yam, carrot and beet) and muscles (working and balancing side masseter and temporalis) averaged. Percent change = 100*((Integrated Voltage $_{Raw\ Food}$ – Integrated Voltage $_{Processed\ Food}$) /Integrated Voltage $_{Raw\ Food}$)). Box plot whiskers extend to the 10th and 90th percentiles.*p ≤ 0.05, non-parametric one-sample sign test.*

Tubers - Masticatory Muscle and Force Calibration.

There was a strong positive association between integrated force (i.e. total force) per bite measured at the first molar, and the balancing-side masseter's integrated EMG voltage (average r^2 0.78±.10, range 0.62 - 0.98). In contrast, the balancing-side masseter's peak EMG voltage explained much less of the variance in peak force per bite (average r^2 0.50±0.16, range 0.27 - 0.85). Peak forces were therefore not calculated, and all of the comparisons (below) were made among integrated forces.

These forces are particularly relevant to mastication because they are the collision impulse, which is a measure of the total amount of energy that can be used to comminute a food item.

Tubers - Masticatory Force. (Table 3.6)

On average, raw tubers were chewed 25.2±9.4 times (Figure 3.4) and required 46.1±26.4 N*s of force per chew (Figure 3.5) and 1105.1±539.7 N*s of total force (Figure 3.6) to masticate completely. Raw carrots were chewed the greatest number of times (27.4±11.1), followed by beets (24.6±9.4) and yams (23.6±7.8). Raw carrots also required the highest average force per chew, 47.4±27.3 N*s, compared to 46.7±26.5 for raw yams and 44.3±27.3 for raw beets, and the highest total chew force (carrot = 1234.3±628.6 N*s; yam = 1058.9±500.9 N*s; beet = 1022.2±529.9 N*s).

On average, slicing the tubers produced no significant effect on the number of chews (26.3±10.3), force used per chew (45.5±27.7 N*s) or total force (1149.4±608.5 N*s). This was true for both the pooled, average tuber and individual (i.e. yam, carrot and beet) tuber comparisons.

In contrast, tenderizing the tubers reduced all force parameters. Compared to raw tubers, chew number was reduced by one chew with tenderizing (24.2±10.3; $p = 0.05$), and both force per chew (41.2±23.5 N*s; $p<0.01$) and total force (973.2±545.5 N*s; $p<0.01$) decreased approximately 11%. Interestingly, there was a great deal of variation in tuber-specific masticatory response to tenderizing. Although the direction of average response was the same for all tubers (i.e. a reduction compared to raw samples), only yams were chewed significantly less when tenderized (21.6±9.1; $p=0.03$). Force per chew, however, was not significantly altered for tenderized yams (43.9 ±25.2 N*s), yet it was lower for both tenderized carrots (42.5±24.9 N*s; $p=0.03$) and beets (874.3±544.2 N*s; $p<0.01$). Tenderization significantly reduced total chewing force used to consume yams (924.8±525.3 N*s; $p=0.02$) and beets (874.3±544.2 N*s; $p=0.02$), but not carrots (1120.4±615.9 N*s).

Compared to tenderizing, roasting tubers further decreased masticatory force parameters. Subjects used an average of three fewer chews when eating roasted (22.4±9.6) versus raw tubers ($p<0.01$), and two fewer chews compared to tenderized samples ($p = 0.02$). Roasting the raw tubers caused a 14% reduction of force used per chew (39.6±24.6 N*s; $p<0.01$), and an approximately 20% decrease of total masticatory force (870.6±489.6 N*s; $p<0.01$), which was also significantly less than the total force used to consume tenderized tubers ($p = 0.04$). As with tenderizing, there was tuber-specific variation in masticatory performance changes resulting from roasting. While chew number, force per chew and total force used to consume roasted yams (17.7±8.0, 34.1±23.2 N*s, and 611.3±445.7 N*s, respectively) and roasted carrots (25.7±10.3, 42.8±24.8 N*s, and 1047.9±535.9 N*s, respectively) was significantly lower than their raw counterparts ($p=0.05$ for roasted versus raw carrots; $p<0.01$ for all other comparisons), roasting beets had no statistically significant effect (chew number = 23.9±9.2; force per chew = 41.9±26.5 N*s; total force = 952.6±529.2 N*s).

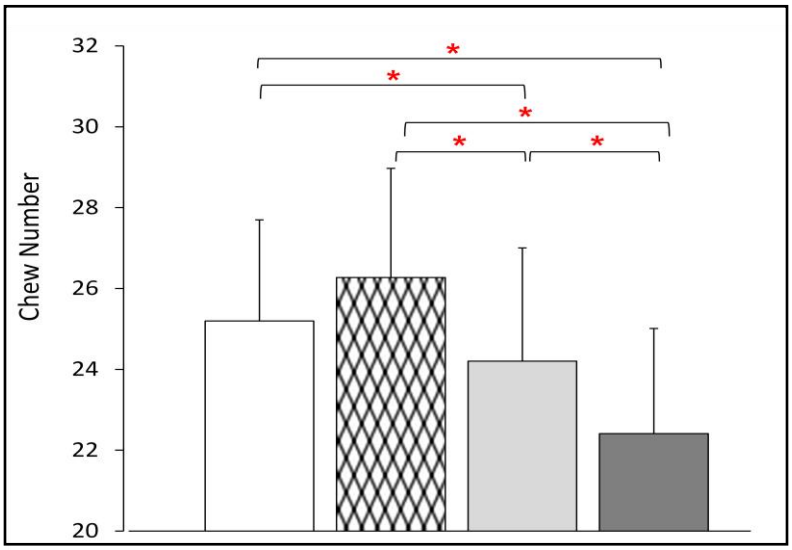

Figure 3.4. *Average number of chews used to masticate size-standardized samples of raw (white), sliced (hatch-mark), tenderized (light grey), and roasted (dark grey) tuber samples. Yams, carrots and beets averaged. *p ≤ 0.05, Wilcoxon signed rank test. Error bars represent ± 1 S.E.*

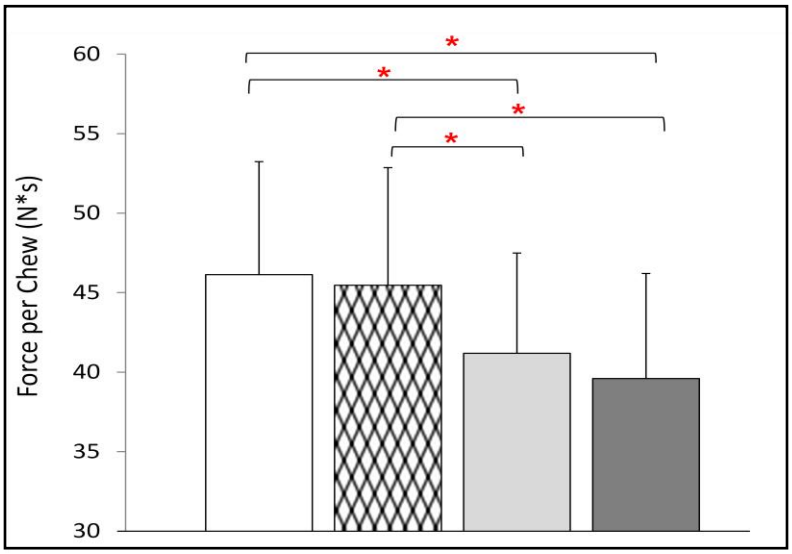

Figure 3.5. *Average force per chew (N*s) used to masticate size-standardized samples of raw (white), sliced (hatch-mark), tenderized (light grey), and roasted (dark grey) tuber samples. Yams, carrots and beets averaged. *p ≤ 0.05, Wilcoxon signed rank test. Error bars represent ± 1 S.E.*

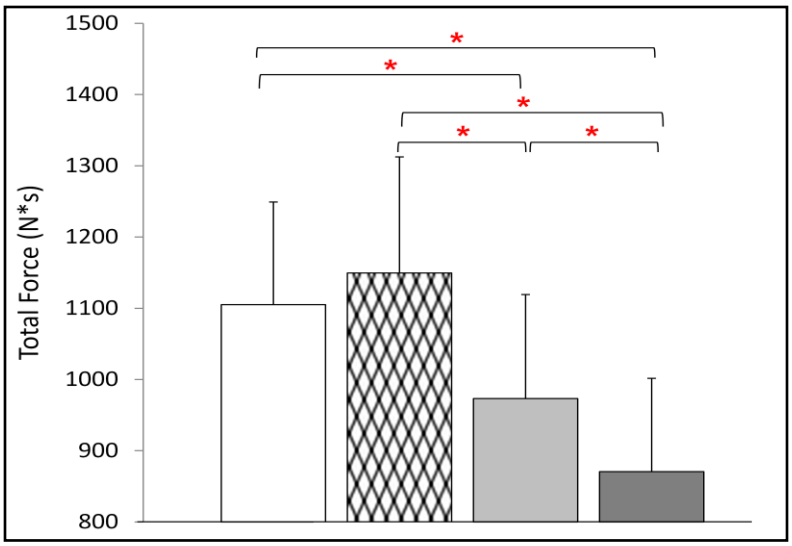

Figure 3.6. *Average total force (number of chews x force per chew, N*s) used to masticate size-standardized samples of raw (white), sliced (hatch-mark), tenderized (light grey), and roasted (dark grey) tuber samples. Yams, carrots and beets averaged. *p ≤ 0.05, Wilcoxon signed rank test. Error bars represent ± 1 S.E.*

Table 3.6. Average number of chews, force per chew and total force used to masticate size-standardized samples of raw and processed tubers. [a]

		Number of Chews	Force *(N*s)*	
			Per Chew	***Total*** [b]
Yam	Raw	23.6 (7.8)	46.7 (26.5)	1058.9 (500.9)
	Sliced	24.4 (9.0)	47.7 (30.7)	1106.4 (573.5)
	Tenderized	21.6 (9.1)	43.9 (25.2)	924.8 (525.3)
	Roasted	17.7 (8.0)	34.1 (23.2)	611.3 (445.7)
Carrot	Raw	27.4 (11.1)	47.4 (27.3)	1234.3 (628.6)
	Sliced	28.2 (11.1)	46.8 (27.5)	1277.5 (679.4)
	Tenderized	27.2 (11.8)	42.5 (24.9)	1120.4 (615.9)
	Roasted	25.7 (10.3)	42.8 (24.8)	1047.9 (535.9)
Beet	Raw	24.6 (9.4)	44.3 (27.3)	1022.2 (529.9)
	Sliced	26.2 (11.0)	42.0 (26.4)	1064.4 (606.6)
	Tenderized	23.8 (9.9)	37.2 (21.4)	874.3 (544.2)
	Roasted	23.9 (9.2)	41.9 (26.5)	952.6 (529.2)
Tuber Average	Raw	25.2 (9.4)	46.1 (26.4)	1105.1 (539.7)
	Sliced	26.3 (10.3)	45.5 (27.7)	1149.4 (608.5)
	Tenderized	24.2 (10.3)	41.2 (23.5)	973.2 (545.5)
	Roasted	22.4 (9.6)	39.6 (24.6)	870.6 (489.6)

[a] *See text for experimental details. One standard deviation in parentheses. Significant differences relative to raw samples are shaded in dark grey, $p \leq 0.05$, Wilcoxon signed rank test. N=14.*

[b] *Total force = average force per chew X number of chews*

Methods and Results: Experiment #2, Comminution (TUBERS)

Methods

Data Collection. 10 adult subjects (5M, 5F) participated in the experiment. Subject age averaged 32±10 years and ranged from 22 to 53 years. All subjects had a complete dental set with the exception of the 3rd molars, which were absent in all but one subject. This male subject possessed four 3rd molars.

Each subject was presented with samples of raw, sliced, tenderized and roasted beets in random order. Beets were selected as the experimental food because their dark color aids image analysis (below). Subjects were instructed to chew the samples five times, twenty times, or until they felt they would typically swallow. After they were done chewing, the subjects spit the comminuted food particles from each trial into plastic vials, rinsing once with a small amount of water to ensure that all of the particles were collected from the oral cavity. Particles were stored in 50% ethanol for no more than eight days before image analysis.

Image Analysis. A sub-sample of food particles from each comminuted food sample was placed into a thin layer of water covering a transparent plastic tray fitted onto the top of an Epson™ perfection v500 flatbed scanner. Each individual food particle was arranged to ensure that none of its edges touched or overlapped other food particles. Once arranged, a grey-scale image of the particles against a white background was scanned at 400 DPI. This process was repeated until all of all of the particles in each sample were scanned.

After scanning, the two-dimensional surface area (mm^2) of each particle was quantified in iVision v4 (BioVision Technologies) using threshold analysis. First, air bubbles and other non-food imperfections in the image were digitally removed. Every colored pixel with a value ranging from 0 to 230 (pure black to very light grey, respectively) was then transformed into the measurement color (green). After thresholding, the image was reviewed and digitally cleaned by hand if needed. The surface

area of every individual food particle was measured by quantifying the number of green pixels comprising the particle (a single particle was defined as the sum of all green pixels in contact). Preliminary tests indicated that thresholding to 230 was the boundary between very small, light particles and shadows resulting from the scanner's moving light source. Particles four pixels or smaller (0.016 mm^2) were removed from the data set to further ensure that only food particles, and not shadow color fluctuations, were measured.

For each sample, the number of particles and average particle area were measured. Total particle area (sum of the surface area of all particles) and the size of the particle at the 25^{th}, 50^{th}, 75^{th}, and 100^{th} (largest particle) percentiles were also noted. Significant differences ($p \leq 0.05$) among raw and processed beets were tested using a Wilcoxon signed rank test.

Precision was quantified by calculating the intraclass correlation coefficient for three sets of comminution tests. First, measurement precision was tested by digitizing and analyzing five repeats of one randomly chosen sample, raw beet chewed 20 times. Second, intra-subject comminution precision was tested by having one subject perform five trial repeats of raw beets chewed 20 times and raw beets chewed until 'swallow'. Finally, the effect of measuring two-dimensional particle surface areas, as opposed to true, three-dimensional size, was quantified by scanning one sample five times (raw beet chewed 20 times), randomly redistributing the particles between each scan.

All calculations were performed in Excel (Microsoft 2007) and StatView statistical package (SAS Institute, version 5.0.1).

Results

After 5 chews, comminuted raw beet samples contained an average of 352.8±182.6 particles (Figure 3.7) comprising a total surface area of 1115.7±213.3mm^2 (Figure 3.8) (Table 3.7). Average particle size was 3.77±1.43mm^2 (Figure 3.9), which was much larger than average 25^{th}, 50^{th}, 75^{th} and

100th (largest particle) percentile particle size (0.05±0.01mm^2, 0.19±0.06mm^2, 1.0±0.3mm^2, and 195.2±103.3mm^2, respectively) (Figure 3.10 and Figure 3.11). Compared to raw beets, sliced beet particles after 5 chews had a significantly larger total surface area (1273.5±146.4mm^2; p = 0.05) and the largest particle was reduced by over 50% (92.3±19.0mm^2; p<0.01). Tenderizing produced a similar effect as slicing. Total particle surface area after 5 chews was 1510.0±114.8mm^2, which was significantly greater than the total surface area of both raw (p<0.01) and sliced (p=0.01) samples. Size of the largest particle (119.1±30.1mm^2) was larger than that of raw beets (p=0.01), but smaller than the sliced samples (p=0.02). Additionally, the number of particles (522.1±157.1) also increased with tenderizing (raw versus tenderized, p=0.01). Similar to slicing and tenderizing, particles from comminuted roasted beets samples after 5 chews had a larger total particle surface area (1364.8±141.7mm^2) than that of raw samples (p=0.04). Average size of the particles in the roasted beet boluses (5.0±1.4mm^2) was also larger than that of the raw (p=0.03), sliced (p=0.03), and tenderized (p<0.01) samples.

After 20 chews, comminuted raw beet samples contained 1479.8±393.8 particles, with a total surface area of 1679.6±420mm^2. Average particle area was 1.2±0.3mm^2, while the 25th, 50th, 75th and 100th (largest particle) percentile particle size was 0.05±0.02mm^2, 0.23±0.08mm^2, 1.1±0.3mm^2, and 33.8±`19.7mm^2, respectively. Roasting was the only form of processing that significantly changed comminution performance. Compared to all other sample types, total particle surface area after 20 chews (2213.7±371.6mm^2), average particle size (1.8±0.5mm^2), and size of the largest particle (54.0±17.1mm^2) increased with roasting (p<0.01 for all comparisons). 75th percentile particle size (1.4±0.3mm^2) also increased, but only compared to that of raw samples (p=0.04).

At 'swallow', comminuted raw beet particle measures were very similar to those resulting from 20 chews, likely because of similar chew number (on average, 24.6 chews were used to consume raw beets (Table 3.5)). By the time the subjects were ready to swallow, the raw sample contained 1434.1±635.2 particles, with a total surface area of 1593.3±423.5mm^2. Average particle area was

1.2±0.4mm^2, while the 25th, 50th, 75th and 100th (largest particle) percentile particle size was 0.05±0.01mm^2, 0.22±0.09mm^2, 1.1±0.3mm^2, and 28.5±`26.4mm^2, respectively. Slicing had no significant effect on any of the particle measures compared to raw samples. Tenderizing, however, increased average particle size to 1.5±0.6mm^2 ($p = 0.03$) and the size of the 75th percentile particle to 1.4±0.4mm^2 ($p= 0.02$). Roasting the beets further increased these measures; average particle size of roasted beets was 2.1±0.6mm^2, larger than the other raw and processed samples ($p<0.01$ for all comparisons). Size of the 50th, 75th and 100th (largest) particles, 0.30±0.08mm^2, 1.8±0.5mm^2 and 57.4±24.9mm^2, respectively, were also significantly larger than that of raw beets (50th percentile $p=0.05$; 75th percentile $p<0.01$; 100th percentile $p=0.04$), sliced beets (75th and 100th percentiles $p<0.01$) and tenderized beets (100th percentile $p<0.01$).

Measurement and intra-subject comminution precision was high, with all intraclass correlation coefficients greater than or equal to 0.94.

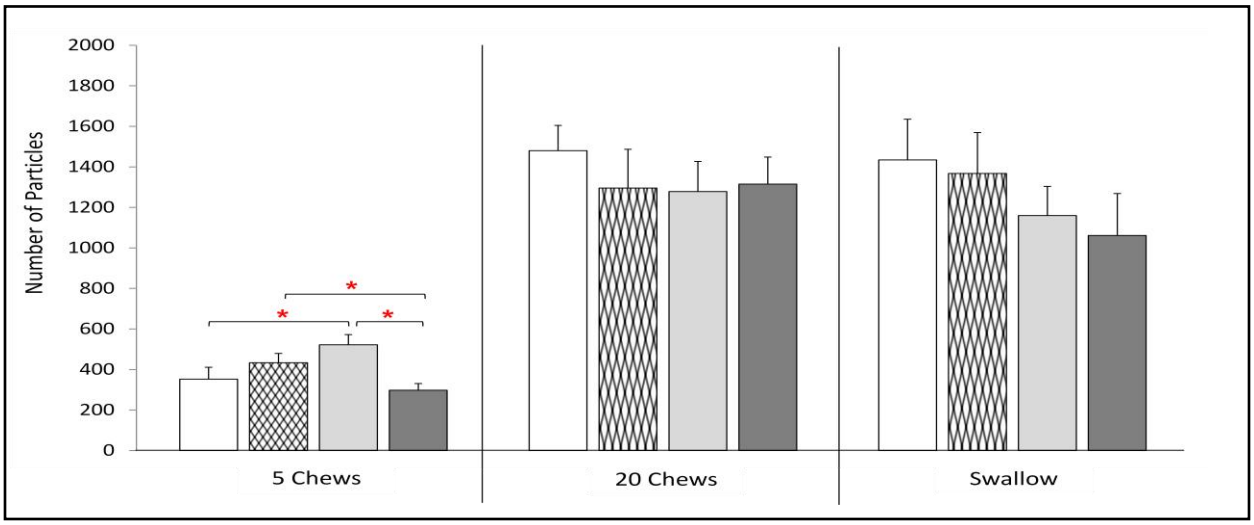

Figure 3.7. *Average number of comminuted food particles resulting from masticating size-standardized raw (white), sliced (hatch-mark), tenderized (light grey), and roasted (dark grey) beet samples for 5 chews, 20 chews or until 'swallow'. *p ≤ 0.05, Wilcoxon signed rank test. Error bars represent ± 1 S.E.*

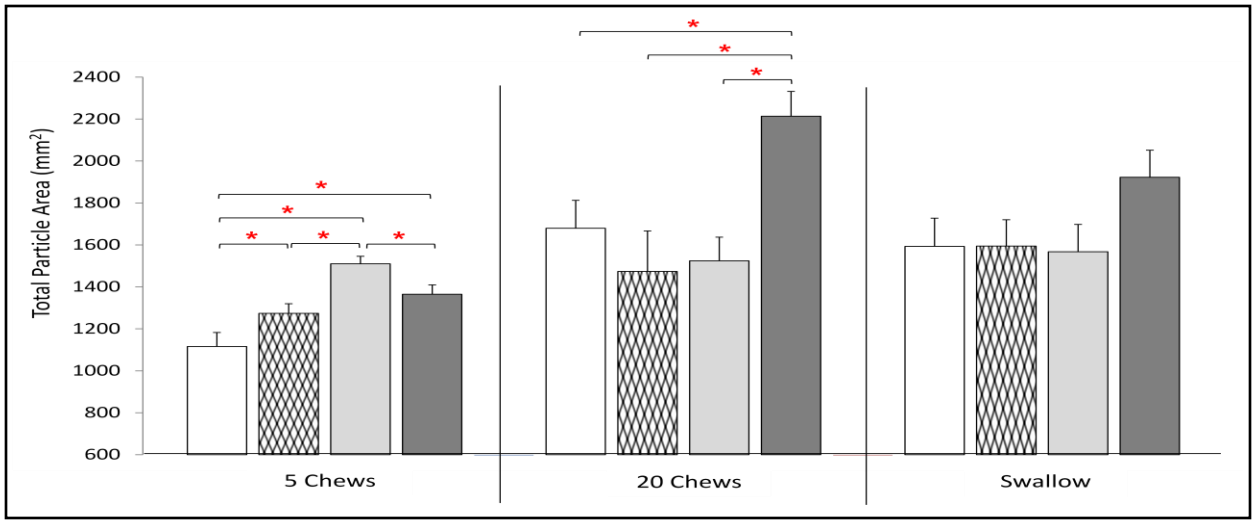

Figure 3.8. *Average total measured area (mm^2) of comminuted food particles (sum of each individual particle area) resulting from masticating size-standardized raw (white), sliced (hatch-mark), tenderized (light grey), and roasted (dark grey) beet samples for 5 chews, 20 chews or until 'swallow'. *p ≤ 0.05, Wilcoxon signed rank test. Error bars represent ± 1 S.E.*

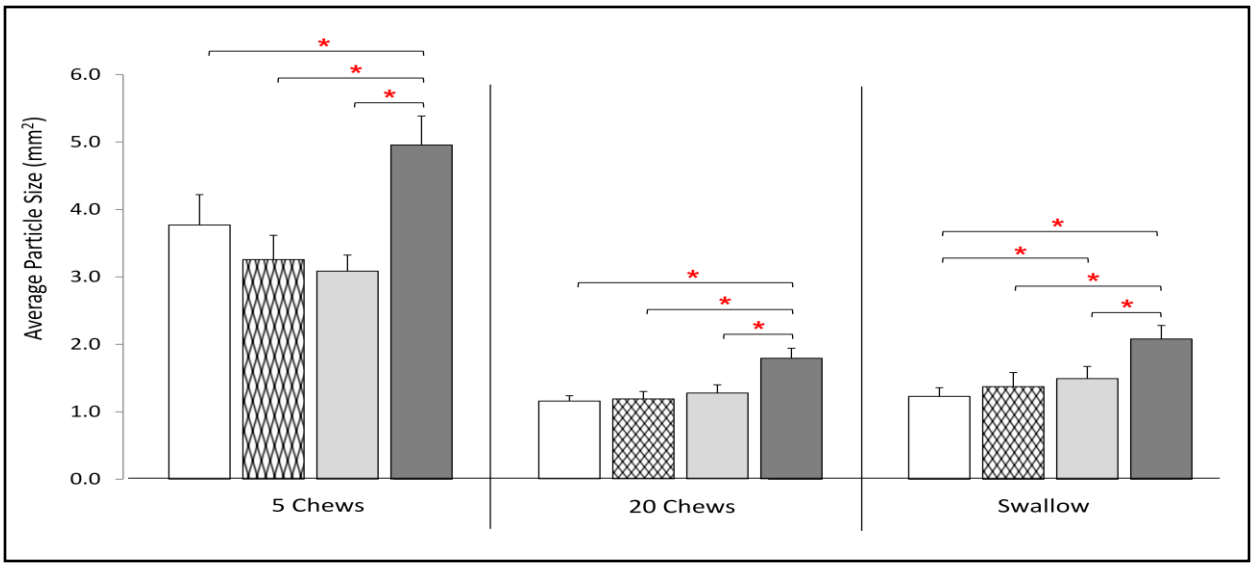

Figure 3.9. *Average size (mm^2) of comminuted food particles (sum of each individual particle area) resulting from masticating size-standardized raw (white), sliced (hatch-mark), tenderized (light grey), and roasted (dark grey) beet samples for 5 chews, 20 chews or until 'swallow'. *p ≤ 0.05, Wilcoxon signed rank test. Error bars represent ± 1 S.E.*

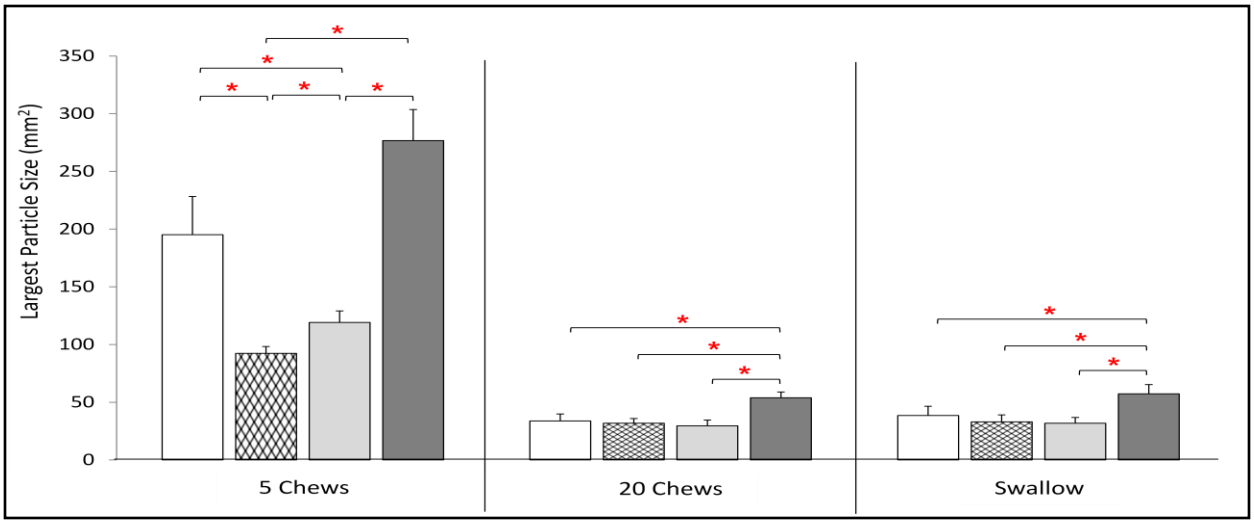

Figure 3.10. *Average size (mm^2) of the largest particle resulting from masticating size-standardized raw (white), sliced (hatch-mark), tenderized (light grey), and roasted (dark grey) beet samples for 5 chews, 20 chews or until 'swallow'. *p ≤ 0.05, Wilcoxon signed rank test. Error bars represent ± 1 S.E.*

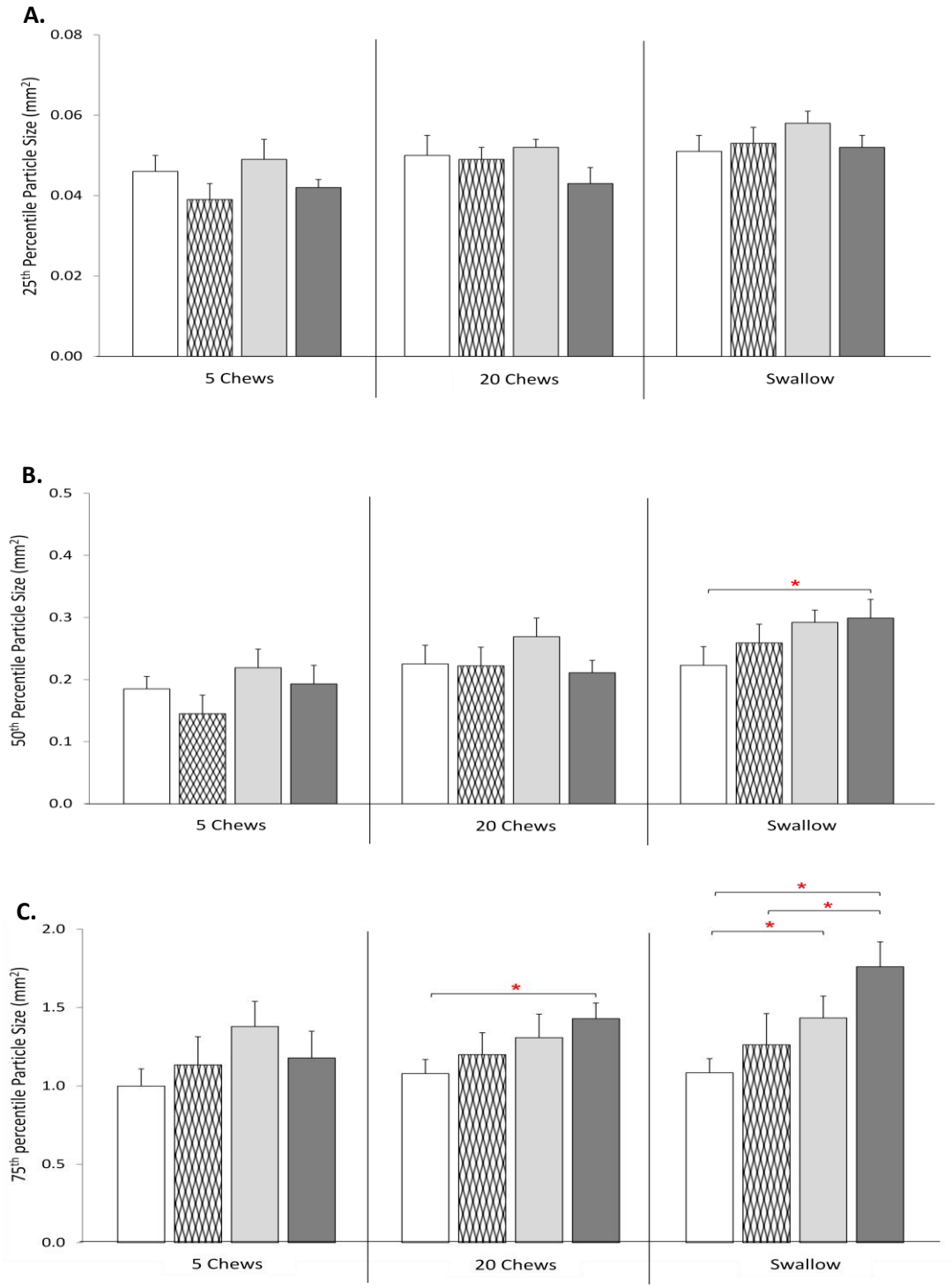

Figure 3.11. *Average 25th (A) , 50th (B) and 75th (C) percentile particle size (mm^2) resulting from masticating size-standardized raw (white), sliced (hatch-mark), tenderized (light grey), and roasted (dark grey) beet samples for 5 chews, 20 chews or until 'swallow'.*p ≤ 0.05, Wilcoxon signed rank test. Error bars represent ± 1 S.E.*

Table 3.7. Measures of food particle number and size (mm^2) resulting from comminuting size-standardized samples of raw and processed beets for 5 chews, 20 chews, and until 'swallow'. [a]

			5 Chews	20 Chews	Swallow
Number of Particles		Raw	352.80 (182.63)	1479.80 (393.84)	1434.10 (635.19)
		Sliced	433.20 (145.50)	1295.10 (606.65)	1367.60 (637.73)
		Tenderized	522.10 (157.06)	1278.50 (467.43)	1159.50 (454.40)
		Roasted	297.70 (103.05)	1315.30 (419.73)	1061.30 (653.39)
Particle Size (mm^2)	Total	Raw	1115.73 (213.29)	1679.62 (419.99)	1593.28 (423.47)
		Sliced	1273.53 (146.39)	1473.79 (609.63)	1594.36 (399.07)
		Tenderized	1510.04 (114.84)	1524.06 (355.86)	1567.62 (412.09)
		Roasted	1364.77 (141.69)	2213.67 (371.60)	1922.87 (406.84)
	Average	Raw	3.77 (1.43)	1.16 (0.25)	1.23 (0.41)
		Sliced	3.26 (1.13)	1.19 (0.35)	1.37 (0.65)
		Tenderized	3.09 (0.76)	1.28 (0.39)	1.49 (0.58)
		Roasted	5.00 (1.37)	1.79 (0.47)	2.08 (0.62)
	Largest	Raw	195.19 (103.26)	33.82 (19.73)	38.52 (26.39)
		Sliced	92.32 (18.99)	31.89 (14.00)	33.05 (17.49)
		Tenderized	119.10 (30.06)	29.65 (14.30)	31.87 (17.04)
		Roasted	276.63 (83.91)	53.95 (17.13)	57.36 (24.90)
	25th Percentile	Raw	0.05 (0.01)	0.05 (0.02)	0.05 (0.01)
		Sliced	0.04 (0.01)	0.05 (0.01)	0.05 (0.01)
		Tenderized	0.05 (0.02)	0.05 (0.01)	0.06 (0.01)
		Roasted	0.04 (0.01)	0.04 (0.01)	0.05 (0.01)
	50th Percentile	Raw	0.19 (0.06)	0.23 (0.08)	0.22 (0.09)
		Sliced	0.15 (0.08)	0.22 (0.08)	0.26 (0.10)
		Tenderized	0.22 (0.10)	0.27 (0.09)	0.29 (0.07)
		Roasted	0.19 (0.09)	0.21 (0.07)	0.30 (0.08)
	75th Percentile	Raw	1.00 (0.33)	1.08 (0.27)	1.08 (0.30)
		Sliced	1.13 (0.57)	1.20 (0.44)	1.26 (0.64)
		Tenderized	1.38 (0.50)	1.31 (0.46)	1.43 (0.43)
		Roasted	1.18 (0.53)	1.43 (0.32)	1.76 (0.51)

[a] *See text for experimental details. One standard deviation in parentheses. Significant differences relative to raw samples are shaded in dark grey, $p \leq 0.05$, Wilcoxon signed rank test. N=10.*

Methods and Results: Experiment #3, Force and Comminution (MEAT)

Methods

Data Collection. 10 adult male subjects participated in the experiment. Subject age averaged 36±17 years and ranged from 23 to 68 years. All subjects had a complete dental set with the exception of the 3rd molars, which were variably present; six subjects lacked third molars, three subjects had all four third molars, and one subject had an incomplete set (the upper left third molar was missing).

Surface electromyographic (EMG) electrodes (Cleartrace™, Conmed Corporation) were affixed to the skin overlying each subject's right and left anterior temporalis and superficial masseter. (One subject had a beard, which did not allow for data to be collected from the masseter muscles.) A ground was placed on the back of the hand. Electrodes were connected to a pre-amplifier and amplifier (MA300™ EMG system, Motion Lab Systems, Inc.) and the analog input converted to digital signals using a PowerLab ™ 16sp A/D board (ADinstruments, Inc.). Data was collected (and viewed) at 1000 Hz in LabChart v7 (ADinstruments, Inc.).

Following EMG electrode placement, the subjects performed an EMG-force calibration trial using a small dime-sized transducer (see Chapter 3 for a description) that was linked to a Kistler™ Dual Mode Amplifier and connected to the EMG A/D board. First, the transducer was acclimated to subject' oral temperature for a minimum of 5 minutes. The transducer was then placed between the upper and lower left first molars and the subjects were instructed to bite down with sub-maximal force and release. EMG activity and transducer output voltage, which was calibrated to known forces ($r^2 = 0.99$), was collected at 1000 Hz and viewed simultaneously in LabChart. Approximately 30 bites were completed. The experimenter viewed the output forces in real time and instructed the subjects to bite harder or softer to ensure that a wide range of force were sampled.

After the calibration trial, the transducer was removed and the subjects were presented with samples of raw and processed meat. Subjects were instructed to chew each sample as normally as possible, with the exception that they chew only on the left side (allowing identification of working and balancing sides), while EMG activity was recorded. The IRB would not allow raw, sliced or tenderized meat to be swallowed. Randomized roasted samples were chewed first. Half of the samples were chewed and swallowed, while the other half were chewed until the subjects felt they would normally 'swallow', but instead of actually doing so, they spit the resulting comminuted food pieces into tubes for particle size analysis. One subject, a vegetarian, declined to swallow any of the roasted samples. After the roasted trials, randomized raw, sliced and tenderized meat samples were chewed until the subject felt they would normally swallow, at which point they stopped chewing, and the food particles were collected into tubes. Trials of each sample type were performed in triplicate. Comminuted food particles were stored in ~50% ethanol for no more than eight days prior to image analysis (described below).

Data Analysis. (Masticatory Force)

Muscle activity was quantified with a custom-written matlab code. Following Lieberman et al. (2006), EMG signals were filtered (Butterworth bandpass filter, 4^{th} order zero-lag with frequency cut offs at 60 and 300Hz), rectified, binned (5 millisecond integral reset), and Thexton's randomization method used to remove background muscle activity (Thexton, 1996). For every chew, maximum amplitude (v) and integrated area (V*s) of each muscle's EMG signal was recorded (Figure 3.1). The number of chews used to consume each sample was counted and total EMG activity was calculated by summing the voltage of all the bites used to consume the food.

Two sets of analyses were performed. With one exception (noted below), all comparisons were made among the non-swallowed samples. Triplicate trials were averaged for each subject. All

calculations were performed in Excel (Microsoft 2007) and StatView statistical package (SAS Institute, version 5.0.1). Significance was set to $p \leq 0.05$.

Analysis #1 (EMG Activity). The effect of processing on masticatory EMG activity was calculated by normalizing each subject's peak and total voltage used to consume processed meat by the voltage used to consume raw meat. This created a percent change in muscle activity, a positive number indicating an increase in activity and a negative number indicating decreased muscle activity.

- percent change = 100*((processed food parameter – raw food parameter) / raw food parameter)

A non-parametric one-sample sign test was used to test whether the sliced, tenderized or roasted values were significantly different from zero (no change). Analyses were performed on each muscle, as well as a pooled sample of all muscles (to assess overall muscle response).

Analysis #2 (Masticatory Force). While EMG data allows for the assessment of individual muscle responses to the consumption of different diets, the amount of chewing force that is generated is more informative for masticatory morphology. Therefore, the EMG data was transformed into masticatory forces using EMG-force calibration equations; for each calibration trial, the subject's balancing-side masseter EMG activity (peak and integrated voltage) was linear regressed onto the resulting bite force (peak and integrated force) (Figure 3.1). The resulting subject-specific regression equations were then used to transform chewing EMG activity into forces. Balancing masseter activity was used because Proeschel and Morneburg (2002) found a significant difference between the EMG-force relationship of dynamic chewing bites and isometric (calibration) bites for all but the balancing masseter. Although they only analyzed peak EMG-force relationship, it is reasonable to predict that the relationship between muscle recruitment and force production should be the same for integrated values.

Within-subject differences in number of chews, force per chew, and total force used to masticate raw and processed meat were tested using a Wilcoxon signed rank test. Comparisons were also made between the real swallow and the chew to 'swallow' (but then spit) roasted meat trials to determine if non-swallowing significantly affected force parameters. Mid-trial swallows, which naturally occur during the consumption of the former trials, were identified by non-uniform patterning of the EMG signal and were omitted from the analysis.

Data Analysis. (Comminution)

Comminuted food particles were dispersed onto a transparent plastic tray fitted onto an Epson™ perfection v500 flatbed scanner. Food particles were arranged so that they did not touch one another and to maximize surface area contact with the tray. Particles were then scanned to create a 400 dpi grey-scale image against a white background. Images were viewed and measured in iVision v4 (BioVision Technologies). The largest particle in each sample was located and its surface area outlined and measured. In some samples, multiple particles had to be measured in order to locate the largest particle. Sample triplicates were averaged and the size of the largest particle in raw and processed comminuted samples was compared using a Wilcoxon signed rank test. One randomly chosen particle (raw meat) was measured five times to quantify measurement precision. All calculations were performed in Excel (Microsoft 2007) and StatView statistical package (SAS Institute, version 5.0.1). Significance was set to $p \leq 0.05$.

RESULTS

Meat - EMG Recruitment. (Table 3.8)

Average muscle recruitment was most affected by slicing raw meat. While average peak voltage per chew did not significantly change (Figure 3.12), average integrated voltage per chew was 12.7±10.1% less when consuming sliced versus raw meat samples (p=0.02) (Figure 3.13). The difference was even greater when comparing total EMG voltage per sample; total peak and integrated voltages were reduced 30.7±30.3% (p=0.02) and 31.8±31.2% (p=0.02), respectively when raw samples were sliced prior to consumption. Neither tenderizing nor low roast temperatures (medium-rare) had a significant effect on any of the measured parameters of average masticatory EMG recruitment. Roasting to higher temperatures (medium-well), however, increased the amount of integrated EMG voltage used per chew by 15.3±18.1% compared to raw samples (p=0.02). The other three parameters (peak voltage per chew and total, and total integrated voltage) were not significantly affected by roasting, regardless of the temperature.

While all muscles had the same average directional response to consuming differently processed foods (i.e. a decrease or increase of EMG voltage), there was substantial individual muscle variation, especially per chew. With slicing, all muscles had a significant reduction of total peak EMG voltage (balancing masseter = 28.6±30.0% decrease; p=0.02) (balancing temporalis = 33.8±29.2% decrease; p=0.02) (working masseter = 28.2±31.3% decrease; p=0.04) (working temporalis = 29.0±36.5%; p=0.04) and total integrated EMG voltage (balancing masseter = 30.7±31.9% decrease; p=0.02) (balancing temporalis = 22.2±30.4% decrease; p=0.02) (working masseter = 29.3±31.3% decrease; p=0.04) (working temporalis = 29.2±36.1% decrease; p=0.04). In contrast, average peak and integrated EMG voltages per chew were significantly reduced for only the balancing side temporalis (peak EMG voltage= 14.9±15.1% reduction; p=0.02) (integrated EMG voltage = 14.3±14.3% reduction; p=0.02), the balancing side masseter (integrated EMG voltage = 11.1±10.3% reduction; p=0.02)), and the working side masseter

(peak EMG voltage = 10.0±11.3% reduction; p=0.04) (integrated EMG voltage = 12.0±9.9% reduction; p=0.04). Tenderizing significantly affected only the average peak voltage of the balancing side masseter (6.1+9.9% increase; p=0.02), while roasting to medium-well increased the average integrated voltage per chew of the working side masseter 17.7±24.3 (p=0.04) and the average peak voltage per chew of the working side temporalis by 17.4±14.5% (p=0.04).

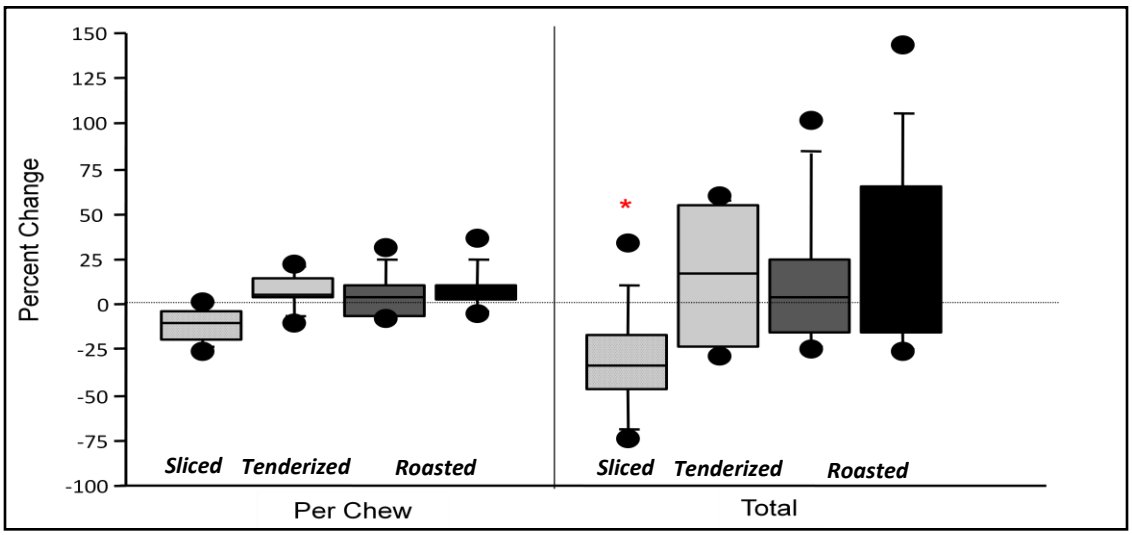

Figure 3.12. *Percent change of peak muscle voltage per chew and total peak voltage (sum of peak voltages per chew) resulting from masticating size-standardized sliced (hatch-mark), tenderized (light grey), medium-rare roasted (MR, dark grey), and medium-well roasted (MW, black) meat, relative to raw samples. Working and balancing side masseter and temporalis responses averaged. Percent change = 100*((Peak Voltage $_{Raw\ Food}$ – Peak Voltage $_{Processed\ Food}$) /Peak Voltage $_{Raw\ Food}$)). Box plot whiskers extend to the 10th and 90th percentiles. *p ≤ 0.05, non-parametric one-sample sign test.*

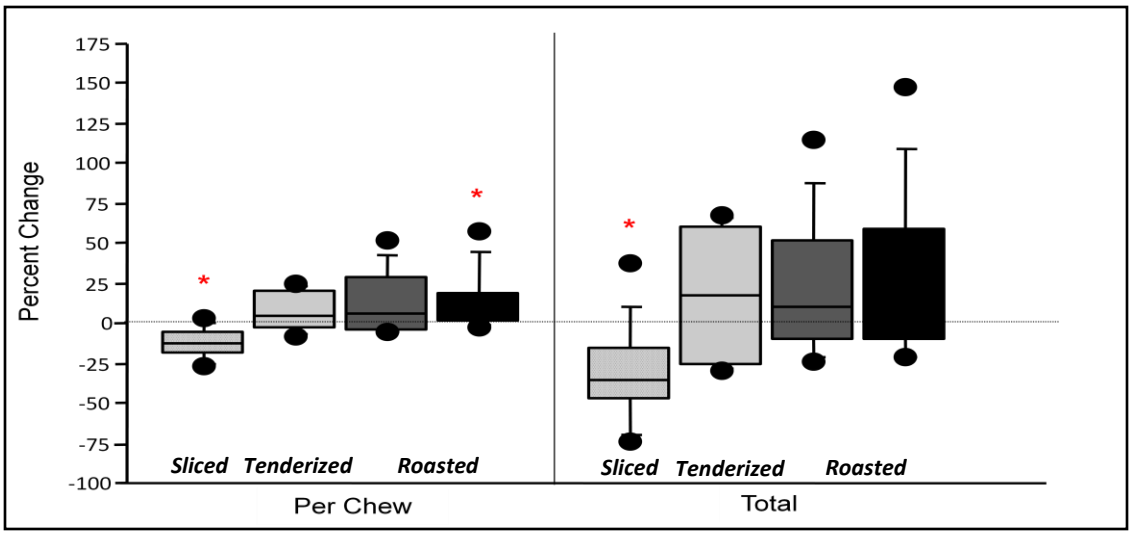

Figure 3.13. *Percent change of integrated muscle voltage per chew and total integrated voltage (sum of integrated voltages per chew) resulting from masticating size-standardized sliced (hatch-mark), tenderized (light grey), medium-rare roasted (dark grey), and medium-well roasted (black) meat, relative to raw samples. Working and balancing side masseter and temporalis responses averaged. Percent change = 100*((Integrated Voltage $_{Raw\ Food}$ – Integrated Voltage $_{Processed\ Food}$) /Integrated Voltage $_{Raw\ Food}$)). Box plot whiskers extend to the 10th and 90th percentiles. *p ≤ 0.05, non-parametric one-sample sign test.*

Table 3.8. Average percent change of muscle recruitment resulting from masticating size-standardized processed tubers, relative to raw samples. [a]

	Peak Voltage per Chew *(v)*					**Total Peak Voltage** [b] *(v)*				
	Balancing Side		**Working Side**		***Muscle Average***	**Balancing Side**		**Working Side**		***Muscle Average***
	Masseter	*Temporalis*	*Masseter*	*Temporalis*		*Masseter*	*Temporalis*	*Masseter*	*Temporalis*	
Sliced	-6.8 (8.9)	-14.9 (15.1)	-10.0 (11.3)	-14.2 (16.3)	-10.6 (9.8)	-28.6 (30.0)	-33.8 (29.2)	-28.2 (31.3)	-29.0 (36.5)	-30.7 (30.3)
Tenderized	6.1 (9.9)	7.0 (13.1)	9.1 (13.2)	8.5 (11.8)	7.2 (10.1)	14.8 (35.0)	16.6 (37.9)	23.2 (36.5)	22.7 (39.2)	16.6 (36.5)
Roasted (MR)[t]	3.2 (9.7)	6.5 (15.2)	5.0 (17.8)	13.7 (23.3)	5.4 (12.4)	11.3 (38.3)	13.1 (35.0)	15.6 (49.1)	25.9 (63.5)	13.9 (41.1)
Roasted (MW)[t]	5.3 (11.9)	7.4 (14.7)	9.8 (18.6)	17.4 (14.5)	8.8 (11.7)	23.0 (51.4)	24.2 (50.7)	30.0 (62.8)	38.3 (60.7)	27.2 (53.7)
	Average Integrated Voltage per Chew *(V*s)*					**Total Integrated Voltage** [b] *(V*s)*				
	Balancing Side		**Working Side**		***Muscle Average***	**Balancing Side**		**Working Side**		***Muscle Average***
	Masseter	*Temporalis*	*Masseter*	*Temporalis*		*Masseter*	*Temporalis*	*Masseter*	*Temporalis*	
Sliced	-11.1 (10.3)	-14.3 (14.3)	-12.0 (9.9)	-13.8 (15.0)	-12.7 (10.1)	-30.7 (31.9)	-33.3 (30.4)	-29.3 (31.3)	-29.2 (36.1)	-31.8 (31.2)
Tenderized	5.2 (11.5)	8.6 (16.5)	8.3 (14.5)	13.5 (12.9)	7.9 (12.6)	15.4 (38.6)	19.8 (44.1)	24.2 (40.2)	28.1 (42.2)	18.7 (40.9)
Roasted (MR) [c]	11.8 (19.3)	11.0 (18.2)	14.3 (24.4)	21.6 (29.5)	12.6 (20.2)	19.1 (39.2)	18.6 (40.5)	24.3 (50.7)	31.9 (63.1)	20.7 (43.3)
Roasted (MW) [c]	12.5 (17.2)	12.0 (18.2)	17.7 (24.3)	24.4 (21.3)	15.3 (18.1)	29.5 (47.8)	28.4 (52.0)	37.4 (61.2)	42.8 (56.9)	32.8 (51.7)

[a] *See text for experimental details. Percent change = 100*((Voltage $_{Raw\ Food}$ – Voltage $_{Processed\ Food}$) /Voltage $_{Raw\ Food}$)). One standard deviation in parentheses.*

Significant changes relative to raw samples are shaded in dark grey, $p \leq 0.05$, non-parametric one-sample sign test. N=10.

[b] *Total voltage = average voltage per chew X number of chews*

[c] *Meat was roasted to medium-rare (MR) and medium-well (MW).*

Meat - Masticatory Muscle and Force Calibration.

There was a strong, positive association between the balancing-side masseter's integrated EMG voltage per bite and the resulting integrated force (average r^2 0.70±0.09, range 0.54 - 0.84). As with the tuber experiment described earlier, peak EMG voltage explained much less of the variance in peak bite force (average r^2 0.38±0.16, range 0.16 - 0.67), and therefore only integrated masticatory forces were compared (below). As mentioned previously, integrated forces are particularly relevant to mastication because they are the force impulse, which is a measure of the total amount of energy that can be used for comminution.

One subject's integrated EMG was a relatively poor predictor of masticatory force (r^2= 0.30), and therefore their force data was omitted from the analyses. This exclusion did not change the significance of the masticatory performance comparisons, but improved the accuracy of the force estimates because the remaining subjects' bite force variance was better explained by their EMG recruitment.

Meat - Masticatory Performance. (Table 3.9)

For all measures of masticatory force performance, there was no significant difference between roasted trials that were actually swallowed and the roasted trials that were stopped when the subjects felt they would normally swallow. All of the comparisons below are made among the latter trials.

On average, 40.1±19.1 chews were used to consume raw meat samples (Figure 3.14). Sliced samples were chewed 31.2±22 times, a reduction of nearly 25% compared to raw samples (p=0.04). Sliced samples were also chewed significantly less than tenderized (42.1±21.7, p<0.01), medium-rare roasted (41.4±18.8, p=0.04), and medium-well roasted (45.3±24.8, p=0.02) samples. Aside from slicing, no other form of processing significantly changed the number of chews used to consume the processed versus raw samples.

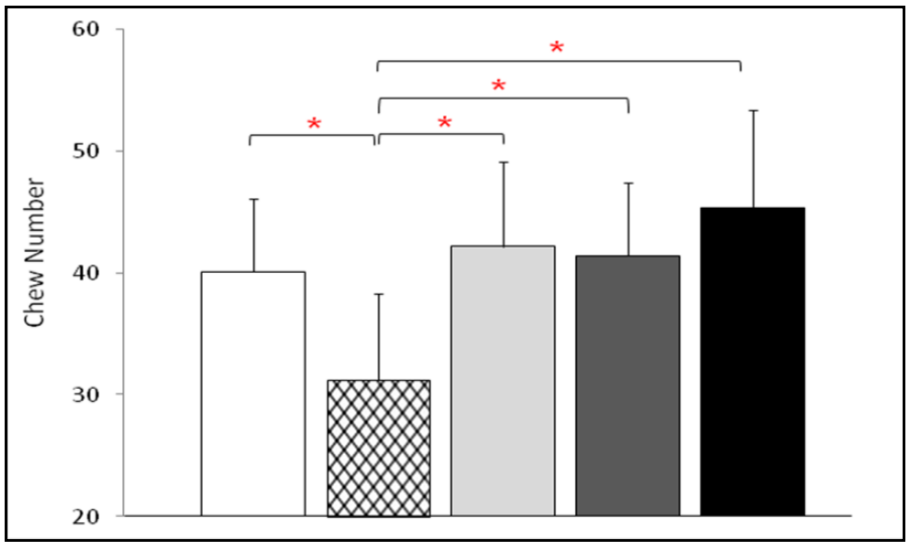

Figure 3.14. *Average number of chews used to masticate size-standardized samples of raw (white), sliced (hatch-mark), tenderized (light grey), and medium-rare roasted (dark grey), and medium-well roasted (black) meat samples. *p ≤ 0.05, Wilcoxon signed rank test. Error bars represent ± 1 S.E.*

As with chew number, average force used per chew was reduced almost 20% when eating sliced versus raw meat (32.7±16.0 N*s and 40.6±21.1 N*s, respectively; p=0.04) (Figure 3.15). In contrast, tenderizing the samples increased force per chew to 47.8±25.2 N*s, nearly 20% higher than average force used per chew of raw meat (p=0.04), and 50% higher than that used for sliced meat (p=0.01). Roasting meat, both to medium-rare and medium-well temperatures, also increased average force per chew compared to sliced samples (50.6±21.9 N*s and 52.2±24.8 N*s for medium-rare and medium-well roasted meat, respectively; p=0.02 for both), but only medium-well roasted meat was significantly increased relative to raw samples (p=0.04).

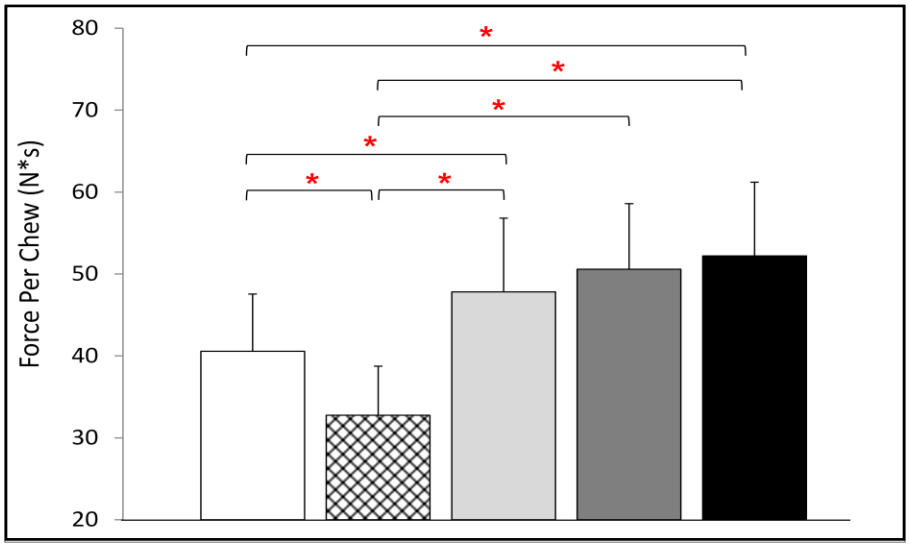

Figure 3.15. *Average force per chew (N*s) used to masticate size-standardized samples of raw (white), sliced (hatch-mark), tenderized (light grey), and medium-rare roasted (dark grey), and medium-well roasted (black) meat samples.*p ≤ 0.05, Wilcoxon signed rank test. Error bars represent ± 1 S.E.*

Total force used to consume sliced (1146.5±1085.7 N*s), tenderized (2157.7±1710.9 N*s), and roasted meat (medium-rare = 1768.8± 787.8 N*s; medium-well = 1848.7±876.0 N*s) did not differ significantly from total force used when eating raw meat samples (1612.6±969.0 N*s) (Figure 3.16). Additionally, processed samples did not differ significantly amongst themselves, with the exception of sliced meat, which required nearly half the force used to consume tenderized meat (p=0.01) and almost 40% less force than medium-well roasted meat (p=0.01).

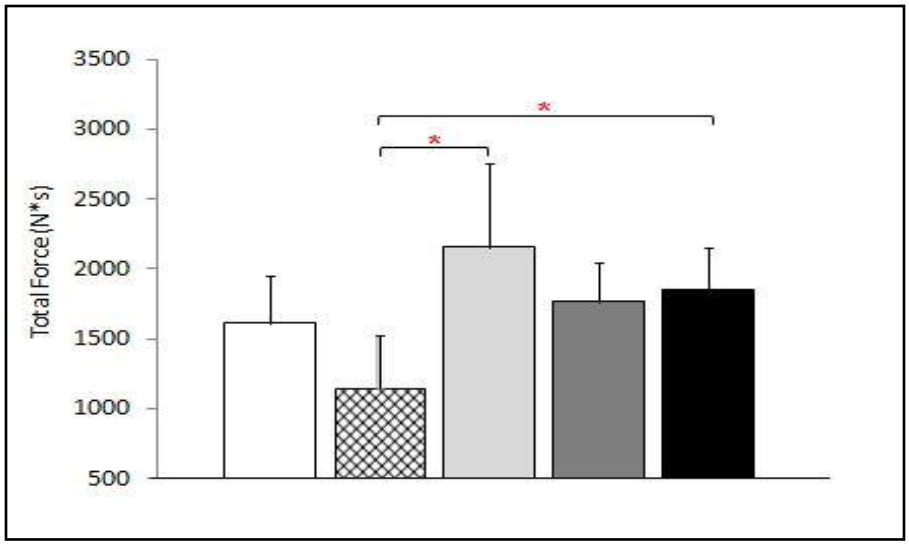

Figure 3.16. *Total force per chew (N*s) used to masticate size-standardized samples of raw (white), sliced (hatch-mark), tenderized (light grey), and medium-rare roasted (dark grey), and medium-well roasted (black) meat samples. *p ≤ 0.05, Wilcoxon signed rank test. Error bars represent ± 1 S.E.*

The size of the largest particle in samples of meat chewed till 'swallow' was reduced by nearly half with slicing (raw = 697.3±207.9 mm^2; sliced = 363.6±108.8 mm^2; p=0.02) (Figure 3.17). A similar reduction in particle size was seen with roasting to medium-well (378.8±183.3 mm^2; p<0.01). Roasting to medium-rare also decreased size of the largest particle, but only to 524.9±218.8mm^2, an approximately 25% reduction compared to raw samples (p=0.01). This effect was much smaller than that of roasting to medium-well (p<0.01). In contrast to the other forms of food processing, tenderizing the meat had no significant effect on size of the largest particle (759.5±136.7mm^2) compared to raw samples, although on average, largest particle size was larger than sliced (p<0.01), medium-rare roasted (p<0.01) and medium-well roasted (p<0.01) samples.

Particle measurement precision was high. The standard deviation of five repeated measurements of the largest particle in one of the raw meat trials was 1.4 mm^2, which was 0.2% of the average area (542.6 mm^2). The maximum difference between any two repeats was 0.5% of the average.

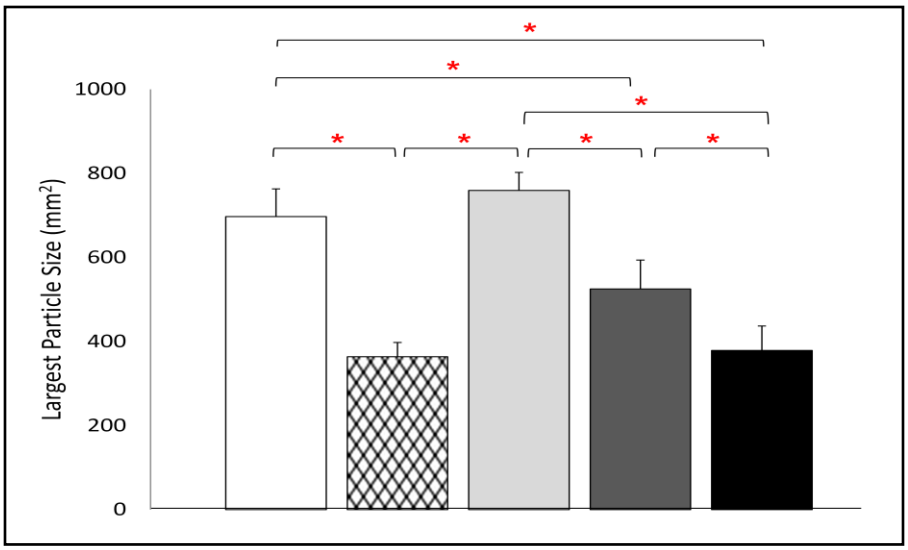

Figure 3.17. *Size (largest two-dimensional surface area) of the largest particle in size-standardized samples of raw (white), sliced (hatch-mark), tenderized (light grey), and medium-rare roasted (dark grey), and medium-well roasted (black) meat samples chewed until subjects felt they would typically 'swallow'. *p ≤ 0.05, Wilcoxon signed rank test. Error bars represent ± 1 S.E.*

Table 3.9. Average number of chews, integrated force per chew and total force used to masticate size-standardized samples of raw and processed meat, and size of the largest particle in the resulting comminuted boluses. [a]

	Size of Largest Particle [d] (mm²)	Number of Chews [d]	Force [e] (N*s)	
			Per Chew	Total [b]
Raw	697.3 (207.9)	40.1 (19.1)	40.6 (21.1)	1612.6 (969.0)
Sliced	363.6 (108.8)	31.2 (22.0)	32.7 (16.0)	1146.5 (1085.7)
Tenderized	759.5 (136.7)	42.1 (21.7)	47.8 (25.2)	2157.7 (1710.9)
Roasted (MR) [c]	524.9 (218.8)	41.4 (18.8)	50.6 (21.9)	1768.8 (787.8)
Roasted (MW) [c]	378.8 (183.3)	45.3 (24.8)	52.2 (24.8)	1848.7 (876.0)

[a] *See text for experimental details. One standard deviation in parentheses. Significant differences relative to raw samples are shaded in dark grey, p ≤ 0.05, Wilcoxon signed rank test.*

[b] *Total force = average force per chew X number of chews*

[c] *Meat was roasted to medium-rare (MR) and medium-well (MW).*

[d] *N=10*

[e] *N=8*

DISCUSSION

Meat and tubers responded to processing in contrasting ways. For tubers, masticatory performance was improved by techniques that altered the food's material properties, such as pounding and roasting. As predicted, pounded tubers required fewer chews than raw tubers, and were chewed with about 11% less force. Roasting further reduced masticatory effort, and compared to raw tubers, roasted tubers were chewed 11% fewer times on average, and with 20% less masticatory force. Unlike pounding and roasting, however, slicing tubers did not alter measured masticatory parameters. This result may stem from the fact that raw tubers readily fracture between human molars and therefore pre-fracturing the food provides little measurable benefit for comminution. It should be noted, however, that only a limited number of domesticated tubers were tested, and it may be that slicing is especially important for tougher foods, such as some wild underground storage organs, which are presumably more difficult to chew raw (Dominy et al., 2008). In addition to slicing, the sharp edges of some Oldowan tools could also have been used to remove the rugged outer peridermal tissue that surrounds the edible portion of many underground storage organs, which could have further aided their consumption by hominins.

In direct contrast to tubers, the comminution of meat was made easier by processing methods that reduce particle size, either by fracturing the food prior to consumption through slicing, or by improving the ability for human low-cusped bunodont molars to create fractures through roasting. Compared to raw meat, approximately 25% fewer chews and 20% less force was used to masticate sliced meat, and size of the largest particle in the resulting 'swallowed' bolus was nearly halved. Roasting to medium-rare did not alter masticatory force or chew number, but did improve the ability of the teeth to effectively comminute the meat, as evinced by a 25% reduction in size of the largest particle within the 'swallowed' bolus. Cooking to a higher internal temperature (medium-well) further aided comminution, and particle size reduction in the bolus was similar to that of sliced meat (approximately

50%). Associated with this increased comminution efficiency, however, was a 30% increase in masticatory force per chew. These masticatory changes are consistent with the material property changes that occur with roasting. In addition to making meat stiffer and tougher, roasting also caused meat to be more brittle and the ratio between toughness and stiffness, $(\text{toughness}/\text{stiffness})^{0.5}$, decreased 30% when meat was roasted to well-done (see Chapter 2 for details). Because raw meat is extremely elastic, making roasted meat more brittle is probably the largest contributor to increased comminution efficiency. In comparison, raw meat does not fracture between the teeth and the 'swallowed' raw meat bolus is composed primarily of a single large particle (Figure 3.18).

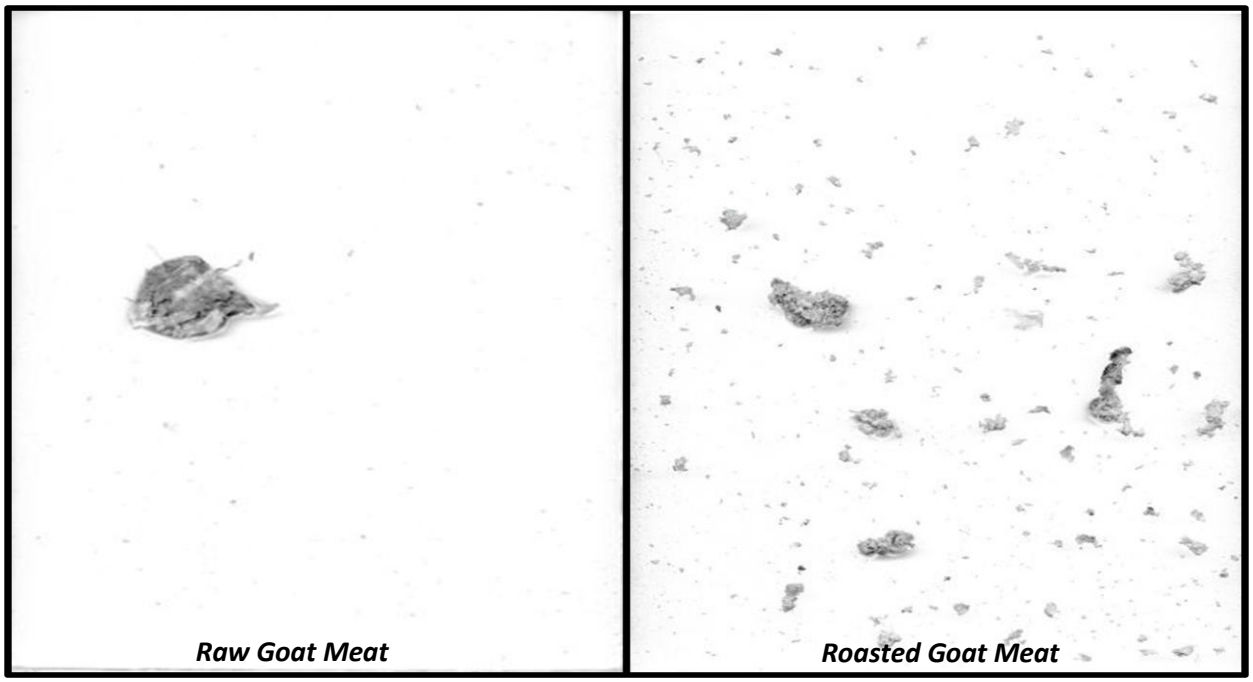

Figure 3.18. *Chewed raw and roasted (medium-well) goat meat bolus at 'swallow'. The individual particles that comprise the bolus have been spread out so that are not in contact with each other.*

The masticatory data from tenderized meat further supports the notion that particle size reduction is particularly important for meat. Pounding with an Oldowan hammerstone mashed and disorganized the muscle fibers, but did not change the meat's toughness (Chapter 2) or fracture it into

multiple pieces. Therefore, as predicted, tenderizing the meat by pounding did not affect chew number, total masticatory force, or intra-oral food fragmentation. Interestingly, however, the force that subjects used per chew when consuming tenderized meat was higher than that of raw meat. One possible reason for this force increase may be the disorganized nature of the muscle fibers after tenderizing. Muscle fibers in raw meat samples primarily run in one uniform direction, and during chewing the teeth preferentially work to separate the weaker perimysial connective tissue that binds the fibers together. Because human teeth are not able to effectively fracture muscle tissue, it is likely that raw meat is mainly indented at the dental cusps and the overall muscle fiber arrangement remains little changed during chewing. Mechanical tenderization, however, caused the muscle fibers to overlap in different directions (like plywood), which may have acted to increase the force of each bite by dampening fracture propagation and requiring more work for indentation/fracture.

Although the masticatory effects of food processing largely conformed to predictions, hypotheses regarding chew number and swallowed particle size for roasted meat and tubers, respectively, were not supported. It was assumed that swallowing of the food would be modulated primarily by particle size reduction and although other factors may come into play, once the food was comminuted to a certain degree (i.e. a certain particle size threshold was reached) a person would swallow. Thus, because the roasted meat was fractured more readily, fewer chews should have been used. This was not the case, however, and roasting had no effect on the number of chews used to consume meat. There are three possible explanations of this result. First, only the size of the largest particle in the comminuted bolus was measured and this may have been a poor proxy of overall comminution efficiency. This is an unlikely confounder, however, as qualitative comparisons of the chewed boluses indicate a clear improvement in comminution when meat is roasted. Most chewed raw meat boluses contained a single large particle and a few smaller particles, while chewed samples of

roasted meat consisted of many more intermediate-sized particles and there tended to be less overall variance in particle size (Figure 3.18).

Another much more likely possibility is that swallowing was modulated by some factor other than particle size. The dual threshold model theorizes that it is the combined effect of particle size and particle lubrication that governs the timing of swallows (Hutchings and Lillford, 1988; Prinz and Lucas, 1995), while in another model the formation of a cohesive bolus is hypothesized to be a primary contributor to the decision to swallow (Prinz and Lucas, 1997; Lucas, 2004). Lubrication and cohesion are both aided by inherent liquid in wet foods, or in the case of dry foods like roasted meat, by saliva, which softens, lubricates and binds the bolus together. Therefore, roasted meat may have been chewed more than what would have been expected based on particle size in order to fully incorporate saliva into the dry bolus.

The dual threshold and cohesive bolus swallowing models may also explain why roasted beets tended to be swallowed at a less fractured state than raw or mechanically processed beets. Compared to the other beet samples, roasted beets are very soft and although some water is lost through evaporation, heating lyses the cells and releases water into the extracellular spaces. Therefore, a less comminuted bolus of roasted beet, as measured by average and median size of the particles, is swallowed because of the freed water, which aids in bolus cohesion and lubrication. Interestingly, however, while roasted beet boluses contain larger particles than those of raw or mechanically processed beets, they also contain approximately 30% more particles, which is a signal of increased comminution efficiency. These opposing responses suggest that roasted beets may macerate in the oral cavity. Smaller particles would be disproportionally affected, which would increase total particle area, while the decreased fracture efficiency would result in larger average particle sizes. It should be noted, however, that the two dimensional measurement of particle size could have biased the results. While particle number will remain the same regardless, differences in fracture pattern amongst the raw and

processed samples could theoretically generate different shaped particles, which may alter the relative measures of two-dimensional sizes. The particles of the raw and processed samples appeared to be broadly similar in shape, however, so it is unlikely that different fracture patterns biased the results (Figure 3.19).

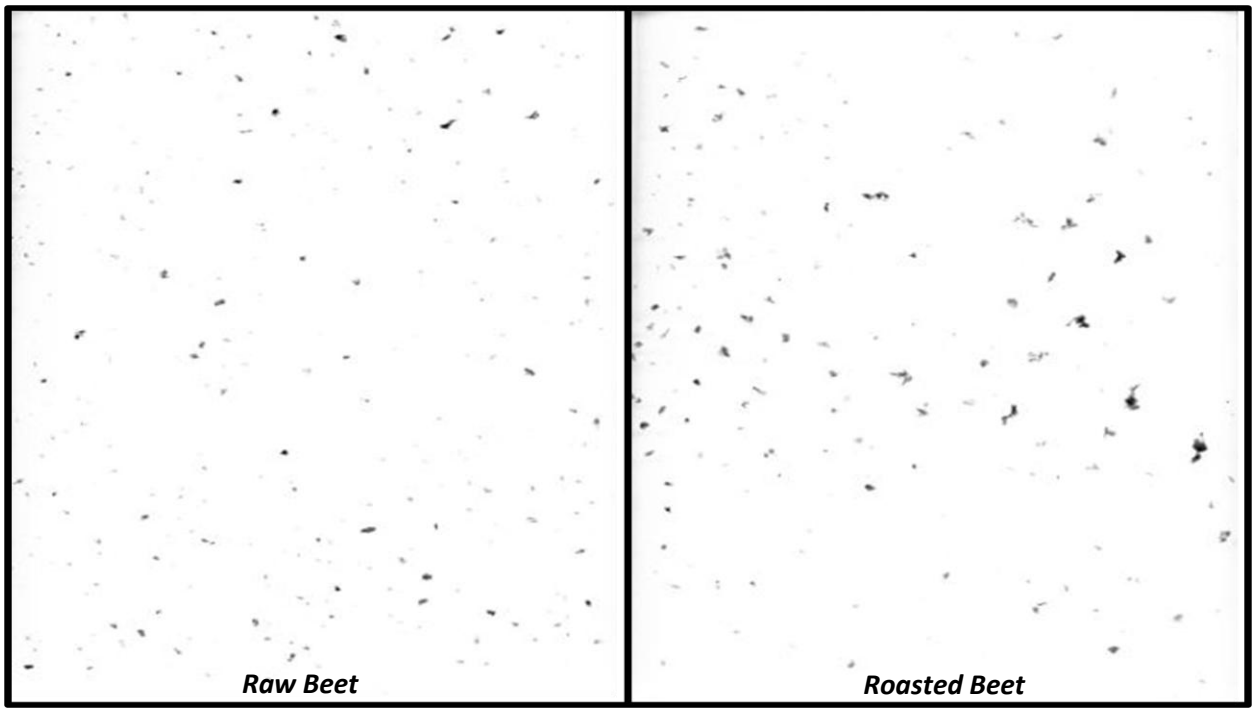

Figure 3.19. *Chewed raw and roasted (15 minutes) beet particles at 'swallow'. The individual particles that comprise a single bolus have been spread out so that they are not in contact with each other.*

Hominin Implications

The contrasting effects of processing on the mastication of meat and tubers highlight the fact that these foods cannot be grouped together when discussing potential hominin masticatory (and morphological) changes resulting from processing. Additionally, because meat and tubers differ from a material standpoint, simply changing the proportions consumed will also affect overall chewing forces. One way to discern the various effects that dietary changes would have had on hominin masticatory

effort, and therefore facial and dental morphology, is to use the data collected in this study to model the masticatory forces that hominins would have generated when consuming different raw and processed diets. While the resulting models (below) are an oversimplification that assumes the consumption of only lean meat and domesticated tubers, they nonetheless provide an opportunity to compare the relative effects of consuming these foods on masticatory effort.

The first step to modeling hominin diets is to calculate the total amount of masticatory force that male and female experimental subjects generated per calorie of raw and processed foods. This was accomplished by dividing the total masticatory force that the subjects generated per gram of tubers and meat by the number of calories (kcal) that the food contained (Table 3.10). Food caloric content was obtained from the USDA National Nutrient Database for Standard Reference (USDA, 2012): kcal per gram- raw yam 1.18; raw beet 0.43; raw carrot 0.41; raw goat meat 1.09; baked yam 1.16; boiled beet 0.44; boiled carrot 0.35; roasted goat 1.43. Sliced and tenderized meat and tubers were assumed to have the same number of calories per gram as their raw counterparts. Caloric data were unavailable for roasted tubers, and therefore boiled or baked tuber values were substituted in the calculation. Yam, carrot and beet data were pooled and the average masticatory force per kcal of tuber was calculated.

- Total Masticatory Force per kcal = $\left[\frac{\text{Total Masticatory Force per Sample}}{\text{Sample Weight}}\right] \div \left[\frac{\text{\# of kcal}}{\text{1 gram}}\right]$

It should be noted that the caloric values used in these calculations are based on the Atwater system, which calculates food energy as the total available energy minus the indigestible components. This system assumes a standard digestibility, however, and also fails to take into account other key variables, such as the cost of digestion, which can significantly alter the net energy gained from a food item. For example, Carmody (2012) found that pounding and roasting meat and sweet potatoes

decreased cost of digestion approximately 9% and 7%, respectively, in rodents. Therefore, the USDA data likely under-reports the energetic gain from processed foods, which will bias the modeled chewing forces; actual masticatory forces per kcal of processed foods will be lower than those calculated in this chapter.

Table 3.10. Average total masticatory force that male and female subjects used to chew raw and processed tubers and goat meat. [a]

			Total Masticatory Force (N*s)			
			Per Sample [b]		Per kcal [c]	
			Male	*Female*	*Male*	*Female*
Tuber	*Raw*		1227.5 (652.6)	982.8 (403.8)	1050.2 (759.9)	807.4 (479.3)
	Mechanically Processed	Sliced	1245.4 (745.1)	1053.6 (443.0)	1067.5 (827.7)	897.2 (557.5)
		Tenderized	1085.6 (676.3)	860.7 (397.6)	954.3 (762.9)	754.3 (495.4)
		Average	1165.5 (707.5)	957.1 (427.1)	1010.9 (788.3)	825.7 (525.9)
	Roasted		1028.0 (622.5)	713.2 (363.5)	855.9 (688.4)	626.1 (476.8)
Meat	*Raw*		1612.6 (969.0)	-	493.1 (296.3)	-
	Mechanically Processed	Sliced	1146.5 (1085.7)	-	350.6 (332.0)	-
		Tenderized	2157.7 (1710.9)	-	659.9 (523.2)	-
		Average	1652.1 (1479.5)	-	505.2 (452.4)	-
	Roasted	Medium-Rare	1768.8 (787.8)	-	412.3 (183.6)	-
		Medium-Well	1848.7 (876.0)	-	430.9 (204.2)	-
		Average	1808.8 (805.9)	-	421.6 (187.9)	-

[a] See text for experimental details. N = 14 and 8 for the tuber and meat experiments, respectively.

[b] The average total masticatory force that each subject generated per sample was calculated. Beet, carrot and yam data were averaged. One standard deviation in parenthesis. Female subjects did not participate in the meat experiment.

[c] The total masticatory force that each subject generated was divided by the number of kilocalories in each food sample. One standard deviation in parenthesis. kcal per gram: raw yam 1.18; raw beet 0.43; raw carrot 0.41; raw goat meat 1.09; cooked yam 1.16; cooked beet 0.44; cooked carrot 0.35; cooked goat 1.43 (USDA, 2012).

After total masticatory force per kcal was calculated, total daily masticatory force was modeled for *H. habilis*, *H. erectus* and *H. sapiens* by assuming that they consumed the number of calories per day estimated in Aiello and Key (2002) (Table 13.11). (N.B., *H. habilis* was modeled as having the same body size and caloric requirements as *A. africanus* because of limited postcranial material and therefore potentially inaccurate body size estimates.) These hominin species represent major morphological transitions within *Homo.* As discussed earlier, *H. habilis* is thought to have been relatively similar to gracile australopiths in post-cranial form, but had slightly smaller postcanines and possessed jaws that were a bit more gracile. *H. erectus,* however, was more derived, with larger bodies and brains (particularly later *H. erectus*), greatly reduced postcanine teeth and probably shorter, less voluminous intestinal tracts, which suggest reduced masticatory and digestive effort. Relative to *H. erectus*, *H. sapiens* had even smaller postcanines, yet had to accommodate the higher caloric demands of a much larger brain.

For each hominin species, total masticatory force was calculated separately for males and lactating females. Lactation is the most energetically costly period in a females life and the calories required during this period provide an upper boundary for daily masticatory force generation. When possible, average masticatory forces from male and female experimental subjects were used to model the total masticatory forces of male and female hominins, respectively. The one exception was the masticatory force used to chew meat. Because females did not participate in the meat experiments, male masticatory data were used to calculate the masticatory force that female hominins would have generated per kcal when consuming meat.

Total masticatory force was calculated assuming that meat contributed a low (5%), medium (25%), or high (50%) proportion of the daily calories, with tubers making up the remaining amount. On average, many modern hunter-gatherer groups obtain approximately 50% of their total daily calories

from meat, although this percentage is slightly lower (approximately 35%) for African bushmen (Kaplan et al., 2000). Consumption of more than 50% meat, especially lean meat that is high in protein but low in fat, was likely unsustainable for hominins because of the high energetic costs associated with digesting proteins, deficiencies in essential fatty acids such as linoleic acid, decreased absorption of calcium, and inability to metabolize amino acids and excrete urea fast enough to maintain the body's acid-base balance (Speth and Spielmann, 1983; Speth, 1989). (N.B., although masticatory force may scale allometrically, total daily masticatory force was not standardized by hominin body mass because the caloric requirements calculated by Aiello and Key (2002) were themselves calculated from body mass estimations, which negates the utility of standardizing by this variable.)

Table 3.11 lists the estimated total masticatory force generated by male and female *H. habilis*, *H. erectus* and *H. sapiens* consuming diets of raw and processed tubers and meat. Three results are immediately apparent. First, regardless of whether the food is raw or processed, simply increasing the proportion of meat in the diet significantly decreases total masticatory force. For example, compared to a low raw meat diet, total masticatory force is reduced by approximately 10% and 20% when consuming a medium and high raw meat diet, respectively.

Second, regardless of the amount of meat consumed, mechanically processing the foods does not affect the amount of total masticatory force generated on average. This assumes, however, that hominins would have sliced and tenderized both meat and tubers, which might not have always been the case. The greatest reduction in masticatory force would occur if hominins consumed tenderized tubers and sliced meat. Compared to a raw food diet, mechanically processing in this manner would decrease total masticatory force approximately 10 to 15%, depending on the amount of meat consumed (greater masticatory reductions occur when consuming greater proportions of meat). In contrast to mechanical processing, roasting meat and tubers clearly reduces masticatory force. Hominins that

roasted their foods would have reduced their total masticatory force production by approximately 20%, and this reduction stays that same regardless of the amount of meat being consumed. This is especially interesting because compared to raw meat, more masticatory force is needed *per chew* when consuming roasted meat. This increased force per chew of roasted meat must be mitigated by fewer overall chews because roasted meat is approximately 30% more calorically dense than raw meat and therefore less of it needs to be consumed to attain the same number of calories.

Finally, regardless of the proportion of meat in the diet or whether the foods were consumed raw or processed, simply because of their larger body size and the need to ingest more calories, the total masticatory force of *H. erectus* is significantly increased relative to smaller-bodied early hominins. Although variation in *H. erectus* body mass must be acknowledged (e.g. Dmanisi *H. erectus* were ~40-50 kg (Lordkipanidze et al., 2007)), the larger bodies of most early and especially later populations of *H. erectus*, would have substantially increased their daily caloric requirements. If diet is held constant, compared to a 30-40 kg australopith or *H. habilis*, a 50-65 kg *H. erectus* would need to generate approximately 40% (male) or 50% (female) more total masticatory force in order to meet the high caloric demands of a larger body. This increases to 50% (male) and 60% (female) more total masticatory force required by the even bigger (~55-70kg) *H. sapiens*. These force increases correspond to *H. erectus* using approximately 8,000 to 11,000 more chews per day than *H. habilis*, with the lowest increase for a roasted, high meat diet and the greatest increase when consuming a raw, low meat diet. Male and female *H. sapiens* would need to chew a further 2,000 to 3,000 more times if consuming the same foods as *H. erectus*.

Table 3.11. The total daily masticatory force generated by hominins. [a]

		Total Masticatory Force (kN*s)					
		Homo habilis		***Homo erectus***		***Homo sapiens***	
		Male	*Female*	*Male*	*Female*	*Male*	*Female*
5% Meat [b] 95% Tuber	Raw	1540.3	1323.1	2133.5	1968.5	2309.0	2109.6
	Mechanically Processed	1484.9	1353.1	2056.9	2013.3	2226.0	2157.5
	Roasted	1256.8	1029.3	1740.9	1531.4	1884.0	1641.1
25% Meat [b] 75% Tuber	Raw	1372.4	1218.0	1901.0	1812.2	2057.3	1942.1
	Mechanically Processed	1332.6	1246.0	1845.8	1853.9	1997.6	1986.7
	Roasted	1125.9	960.9	1559.6	1429.7	1687.8	1532.1
50% Meat [b] 50% Tuber	Raw	1162.6	1086.7	1610.4	1616.8	1742.8	1732.7
	Mechanically Processed	1142.1	1112.1	1582.0	1654.6	1712.1	1773.2
	Roasted	962.3	875.5	1333.0	1302.6	1442.6	1395.9

[a] *Estimated total masticatory force (kN*s) that H. habilis, H. erectus and H. sapiens would generate per day if they consumed a raw, mechanically processed (average of sliced and tenderized data) or roasted diet of tubers (yams, carrots and beets) and meat. Forces were calculated assuming that the taxa must meet the daily caloric requirements estimated by Aiello and Key (2002). Estimates for lactating females were calculated because this is the most energetically costly period for females: H. habilis (estimated to have a similar body mass as A. africanus) male = 1506.6 kcal, lactating female = 1671.2 kcal; H. erectus male = 2086.9 kcal, lactating female = 2486.5 kcal; H. sapiens male = 2258.5 kcal, lactating female = 2664.7 kcal.*

[b] *Meat comprised a low (5%), medium (25%) and high (50%) proportion of the total daily calories.*

In contrast to total masticatory force, which will affect both overall facial size and robustness of the bones, peak masticatory force per chew may best inform tooth size. Bite forces are distributed across the occlusal surface area and dental stress (force per occlusal area) is a main contributor to food fracture. Eng et al. (2013 (in review)) calculated maximum bite force at the second molar in macaques,

apes and hominins, and found that dental stress remained fairly constant ($r^2 = 0.93$), with two exceptions; *Homo* species and to a much lesser extent *A. africanus*, were not able to generate the amount of maximum bite force that is predicted from postcanine tooth size (Figure 3.20). These data indicate a grade shift with the genus *Homo* whereby the foods that they consumed did not require high dental stress to fracture.

One possible explanation for reduced occlusal stress in *Homo* species is the consumption of processed foods. Although peak masticatory forces were not quantified in this dissertation, comparisons can be made based on peak masticatory muscle EMG data (Figures 3.1 and 3.11), which will be related to peak force production (Proeschel and Morneburg, 2002). While analysis of this data shows no effect of processing on the peak EMG of subjects consuming meat or mechanically processed tubers, average peak muscular recruitment when consuming roasted tubers decreased 14% compared to raw tubers. These data suggest that the ability of *Homo* to roast tubers may have been especially important in allowing for the reductions of peak chew force and dental size seen within the genus.

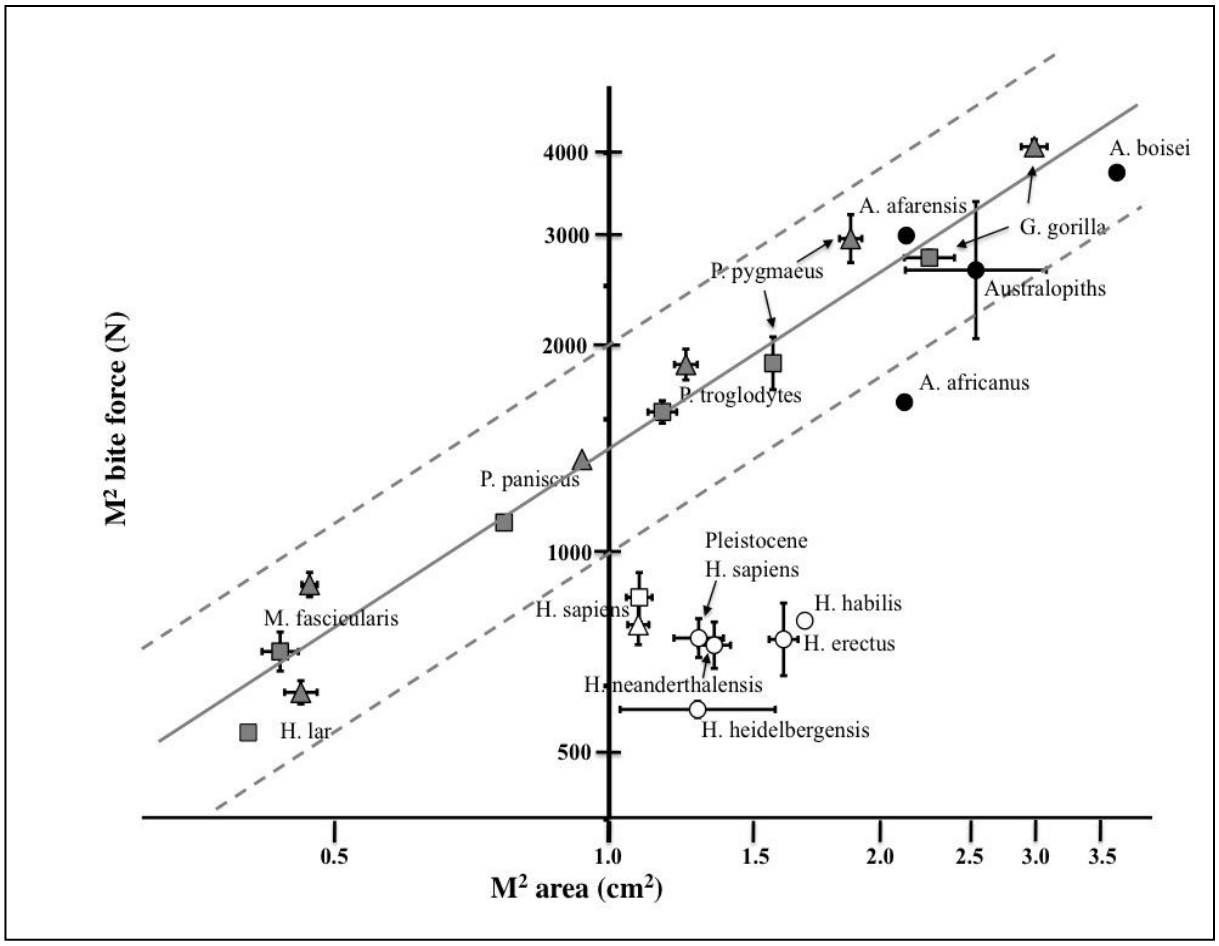

Figure 3.20. *Logarithmic plot of maximum bite force at the second molar and second molar occlusal area (from Eng et al. (2013 (in review))). Bite force was calculated using lever mechanics and skeletal proxies of maximum masticatory force generation. Grey symbols = Extant nonhuman primates; Black symbols = Australopithecines; White symbols = Homo species. Males and females of extant species are designated by triangles and squares, respectively. All other taxa are represented by circles. The solid line represents the regression of bite force against second molar area, excluding Homo, and the dashed lines are the 95% prediction intervals (r^2 = 0.93).*

Although the meat and tuber EMG data cannot be directly compared because the data were collected using two different experimental protocols and the EMG data were uncalibrated, it is unlikely

that increasing the amount of meat in the diet of *Homo* would have lowered peak masticatory forces. A gram of raw meat requires an average of 537.5 N*s of force to consume and this is very similar to the 544.5 N*s of force used to chew a gram of raw tuber. Additionally, with the exception of sliced meat, which requires 382.2 N*s of masticatory force per gram of food, meat requires either the same or more force to chew than raw or processed tubers. Although future experiments are needed to confirm whether or not total masticatory force generated per gram of food can be extrapolated to comparisons of peak chewing forces, the data collected in this chapter suggest that the inclusion of meat in the diet would have only resulted in peak masticatory force reductions if it was sliced prior to consumption. Maximum bite force capabilities, however, are influenced by the most mechanically demanding foods that a species consumes and although chew force was similar for meat and (domesticated) tubers in this study, it is very likely that wild underground storage organs or other tough plant fallback foods would require substantially higher forces per chew than meat, regardless of whether it is raw or processed. If this is true, the lower maximum bite forces generated by species in the genus *Homo* are most likely attributable to processing methods, such as roasting, which reduce the peak chewing forces used to masticate plant foods.

The reduced ability of *Homo* species to produce occlusal stress suggests that while selection may have acted quickly to reduce the size of masticatory musculature, perhaps because it is relatively energetically expensive to maintain, dental size was much less affected by diet. Why was this the case? One possibility is selection was no longer acting on the ability of *Homo* to generate occlusal stress, and instead smaller teeth were selected for other reasons, such as fitting in shorter, less prognathic faces (Lieberman, 2011). Relaxed selection for large, thick enameled teeth may also explain the wide degree of variation in average and relative enamel thickness of *Homo* species (Smith et al., 2012).

The data presented in Eng et al. (2013 (in review)) suggest that unlike what was hypothesized in Chapter 2, the smaller teeth of *H. sapiens* compared to *H. habilis* and *H. erectus* would not have been "allowed" by further reductions in maximum force per chew, because relative to other hominoids, all *Homo* species produce relatively low dental stress. Rather, instead of dental size decreases, overall facial size and robustness of the jaws may be a better indicator of total masticatory effort. If this is true, then the morphological changes evident in the genus *Homo* suggest at least a three phase shift in dietary effort. First, compared to gracile australopiths, *H. habilis* consumed foods that required less peak bite force, which the data collected in this chapter suggests may have been accomplished by roasting tubers and other plant foods. Interestingly, although stone tools pre-date the genus *Homo*, this study did not find any effect of mechanical processing on peak masticatory effort. This may have been due to the small number of processing types and foods tested, however, which will have to be remedied in future studies (see below).

The second major shift in masticatory effort occurred with the evolution of *H. erectus*. Although maximum bite force was the same as in *H. habilis*, the much larger bodies of (most) *H. erectus* would have necessitated the intake of significantly more calories, which might have increased total masticatory force generation by 40-50%. It is likely, however, that high total masticatory forces were mitigated at least in part by consuming a more high-value, energetically dense diet that required less masticatory force per calorie. One very likely possibility is that *H. erectus* consumed more animal-based foods than earlier hominids (e.g., (Hill, 1982; Shipman, 1986; Milton, 1999; Stanford and Bunn, 2001; Bunn, 2007)), which would have decreased total masticatory force, especially if soft fats and bone marrow were consumed in addition to meat. Additionally, the development of the Acheulean industry approximately 1.7 mya (Klein, 2009) may indicate the use of more complex mechanical processing techniques that could have further reduced overall masticatory forces relative to *H. habilis*. Finally, along with decreasing peak chew force, cooking may have also increased the quality of the foods and enabled the

smaller intestines, and larger brains and bodies of *H. erectus* (e.g. (Aiello and Wheeler, 1995; Wrangham et al., 1999; Wrangham, 2009)). Heating "pre-digests" food and increases net energy gain (e.g.(Boback et al., 2007; Carmody and Wrangham, 2009; Carmody et al., 2011)) by denaturing and degrading proteins and cell membranes in meat (Lewis and Purslow, 1989; Tornberg, 2005), and hydrolyzing pectin substances, cell walls, gelatinizing starch and reducing intercellular adhesion in plant material (Greve et al., 1994a; Ng and Waldron, 1997; Alvarez and Canet, 2001; Lillford, 2001). Further energetic savings may result from increased comminution efficiency, such as when meat is sliced or roasted, or the use of processing techniques that significantly reduce average size of ingested food particles (i.e. grinding). These processing methods create a more fractured food bolus, which aids food digestion by increasing the surface area that comes into contact with digestive enzymes, thereby reducing digestive times and energetic costs associated with digestion.

The final major shift in masticatory effort, the consumption of foods that required low maximum bite forces as well as substantially lower total masticatory forces occurred with *H. sapiens*. Although some of the reductions in *H. sapiens*' facial robusticity may be caused by reduced prognathism, which increases the effective mechanical advantage of the masticatory system and causes higher bite forces and lower bone stresses to be generated for a given amount of muscular force (Wroe et al., 2010), the overall gracility of the face of modern *H. sapiens* in particular argues for an overall reduction in masticatory effort. The hypothesis needs to be tested, but it is possible that a secular trend in reduced masticatory effort first began in *H. sapiens* with new processing techniques associated with the Middle Paleolithic, continued through the formation of specialized, more effective tools in the Upper Paleolithic, and then reached its pinnacle in modern food processing techniques and tools, such as electric blenders, microwaves, canning, and complex pre-processing methods, which have almost entirely removed the masticatory forces that modern *H. sapiens* generate.

Future Studies

Future work should focus on three main areas. First, kinematic changes associated with food processing should be analyzed. While vertical forces provide most of the energy necessary for comminution, there is also a smaller horizontal force component that aids in food fracture. It is necessary to quantify this force because there is evidence to suggest that changing the material properties of the diet (as with processing) will affect jaw patterns and therefore the angle at which the force is applied in the food bolus. For example, Reed and Ross (2010) found that *Cebus* monkeys used less lateral jaw excursions when chewing tougher foods. Additionally, the experiments within this dissertation chapter demonstrated a differential effect of processing on the activity of the four masticatory muscles (Table 3.4 and Table 3.8). For example, compared to when raw meat was chewed, the average muscular recruitment when consuming sliced meat was significantly reduced for all muscles except the working side temporalis muscle, which exhibited no significant change in activity. This non-uniform response suggests that jaw movement patterns, and therefore horizontal force production, was altered. By analyzing kinematic patterns it may be possible to estimate changes to these horizontal forces.

Second, the effect of food processing on peak, not just integrated forces, should be investigated. As discussed previously, peak forces are especially relevant to dental size and enamel. Unfortunately, peak forces are poorly estimated by EMG-force calibrations like those used in this study (r^2 ranged from 0.16 to 0.85). Future projects will need to improve on the EMG-force calibrations, possibly by using a more dynamic chewing bite as opposed to an isometric clenching bite during the calibration. Another alternative is to use a dental force transducer to measure *in vivo* chewing forces (e.g.,(Hagberg, 1987; Proschel and Raum, 2001; Shimada et al., 2012)). The problem with this method, however, is that the

size of the transducer and its wires precludes 'normal' chewing and may confound the results. The data could be used, however, to corroborate EMG data.

Finally, future experiments should focus on testing more foods and processing types, which will allow for a deeper understanding of the diverse effects that dietary changes would have on masticatory performance and ultimately the morphology of the face and teeth. In fact, the small number of foods and processing techniques analyzed is the main limitation of this study. Other techniques such as grinding, boiling, maceration, drying, etc., would also have been extremely important for hominins, and none of these methods are mutually exclusive. For example, grinding lean dried meat and mixing the resulting powder with equal parts liquid fat forms pemmican, an energetically dense food source utilized by many North American Indians. Furthermore, even when the same processing method is used, not all foods respond in a similar manner, as demonstrated in this chapter by the contrasting effects of roasting on the masticatory force used to chew tubers and meat. Similar differences can also occur within a broad food type (i.e. vegetables and meat). Lucas (2004) found that boiling reduced the toughness of potatoes and white onions, but the same treatment had no effect on white turnips or Chinese leaf vegetables. Additionally, because of variations in collagen and fat distribution, meat from different body regions and animals will also respond differently to processing treatments (Wheeler et al., 2000; Schonfeldt and Strydom, 2011; Dixon et al., 2012). It should also be noted that only lean meat was tested in this study, and while muscle tissue is high in protein and calorically dense compared to tubers and many other plant foods, fat deposits, bone marrow and brains may have also been particularly important sources of nutrients available to hunting or scavenging hominins. Stone tools would be needed to remove these foods from muscles, bones and skulls, but once extracted, these soft foods would require little effort to masticate.

Unfortunately, masticatory performance experiments are time and cost-intensive, which severely limits the number of experiments that can be performed. A less costly alternative, however, is to measure the material properties of the foods and use those to predict masticatory effort used to chew the foods. A number of studies have shown that food properties will affect masticatory parameters such as jaw movement and muscle activity, etc. (e.g., (Mioche and Peyron, 1995; Hiiemae et al., 1996; Agrawal et al., 1997; Agrawal et al., 1998; Mioche et al., 1999; Kohyama et al., 2004a; Kohyama et al., 2007; Kohyama et al., 2008; Xu et al., 2008; Reed and Ross, 2010)), but none of these experiments have focused on the correlation between food material properties and masticatory force (which is more useful when modeling diets), or analyzed how the material property changes resulting from processing relate to masticatory force parameters. Therefore, to address this deficit, the masticatory data collected in this chapter was coupled with the food material properties quantified in Chapter 2 in order to determine if, and with what degree of confidence, the average masticatory effort used to chew a standardized piece of food can be predicted from the properties studied in Agrawal et al. (1997, 1998), i.e., stiffness, toughness, (toughness X stiffness)$^{0.5}$, and (toughness/stiffness)$^{0.5}$. Unfortunately, only the relationship for tubers could be analyzed because the meat experiment used different sized samples and the resulting masticatory parameters are not directly comparable to those from the tuber experiments.

The results of the masticatory force and material property regressions are shown in Table 3.12. Tubers are a 'force-limited' food and as one might predict, both stiffness and (stiffness X toughness)$^{0.5}$ explained a large proportion of the difference in chew number, force per chew, and total masticatory force used to consume tubers. In contrast, toughness and (stiffness/ toughness)$^{0.5}$ were much less predictive for force per chew and total masticatory force, and neither of these material properties were significantly correlated to the number of chews that a subject used.

Although these data may not extrapolate as well to 'space-limited' foods, such as meat, and more studies are needed to confirm that these relationships are similar in a wider variety of 'force-limited' foods (e.g. wild tubers, nuts, etc.), the results of these regressions highlight the predictive power of material properties for masticatory parameters, and confirm the utility of using these properties for estimations of hominin masticatory force. Material properties are relatively easy to assess on a broad number of foods and the large number of foods and processing techniques that can be tested will further elucidate the effects that food processing and other dietary changes would have had on hominin mastication.

Table 3.12. Linear regression of masticatory performance variables on tuber material properties (listed below). [a]

		Toughness *(J/m^2)*	**Stiffness** [b] *(kPa)*	**(Toughness x Stiffness)$^{0.5}$**	**(Toughness/Stiffness)$^{0.5}$**
	Raw Beet	1173.8	4372.7	2265.6	0.52
	Sliced Beet	1173.8	4372.7	2265.6	0.52
	Tenderized Beet	322.2	-	-	-
	Roasted Beet	622.6	3069.5	1382.4	0.45
	Raw Carrot	1071.7	6007.3	2537.4	0.42
	Sliced Carrot	1071.7	6007.3	2537.4	0.42
	Tenderized Carrot	769.1	-	-	-
	Roasted Carrot	553.0	4160.8	1516.9	0.36
	Raw Yam	926.6	5081.9	2169.9	0.43
	Sliced Yam	926.6	5081.9	2169.9	0.43
	Tenderized Yam	773.6	-	-	
	Roasted Yam	403.0	1227.5	703.4	0.57
R-Squared [c]	**Number of Chews**	*non-significant*	***0.77 (+)***	***0.70 (+)***	*non-significant*
	Force per Chew *(N*s)*	***0.56 (+)***	***0.90 (+)***	***0.76 (+)***	***0.47 (-)***
	Total Force *(N*s)*	***0.50 (+)***	***0.95 (+)***	***0.82 (+)***	***0.46 (-)***

[a] *See text for experimental details.*

[b] *Stiffness (elastic modulus) at 20%, 40%, 60% and 80% fracture stress was averaged.*

[c] *Only significant r^2 values shown, $p \leq 0.05$. R-squared values significant at the $p \leq 0.01$ level are represented by a dashed underlined, while values significant at the $p \leq 0.001$ level are represented by a solid black underlined. Positive (+) or negative (-) associations indicated in parentheses.*

CHAPTER 4. INTEGRATION

INTRODUCTION

Since the classic work of Olson and Miller (1958), it has been well known that the mammalian masticatory complex is highly integrated, in part through multiple epigenetic interactions, some of which derive from masticatory forces themselves. Morphological integration, or the covariance of structures in a population, has been well characterized in the skull (e.g., (Marroig and Cheverud, 2001; Hallgrimsson et al., 2007; Mitteroecker and Bookstein, 2008; Gkantidis and Halazonetis, 2011; Singh et al., 2012)). Functional integration, which occurs when the morphologies of multiple structures affect their joint performance, is less well studied and is especially pertinent to the study of hominin masticatory morphology; although masticatory forces have a critical role in the cranio-facial morphology and integration of all mammals, the interaction between force and morphology may have been uniquely and exceptionally important in human evolution because of the biomechanical consequences of cooking and other food processing techniques. At tooth-food-tooth contact, the cusps of the mandibular postcanines must fit into the cuspal basins and spaces between the corresponding maxillary teeth (and *vice versa*). Consequently, for efficient comminution to occur, the size, shape and positions of the upper and lower teeth must be properly aligned and there can be no major dental rotations, gaps, dental crowding or impactions. If forces affect the performance and development of the masticatory complex, then function itself must play some role in the overall integration of the system.

In modern western societies occlusal health is markedly poor. For example, close to 50% of the population in the U.S. is afflicted by moderate to major dental problems such as tooth displacements, dental rotations, overjets, and openbites (Kelly and Harvey, 1977). These occlusal variations underlie what happens when normal integrative processes fail, and are not present at such levels in non-industrial populations or wild primates (Mills, 1963; Corruccini, 1984; Corruccini, 1999; Evensen and Øgaard, 2007). What is the cause of this recent malocclusion epidemic? One of the most pervasive hypotheses in the anthropological literature involves the hypothesis of 'disuse' (see (Corruccini, 1999;

Lieberman, 2011) for review). According to this hypothesis, chewing soft, highly processed food does not produce the stresses necessary to stimulate proper growth and alignment of the jaws and dentition. This hypothesis is supported by relatively low heritabilities for cranio-dental features and measures of dental misalignments (Boraas et al., 1988; Cassidy et al., 1998; Hughes et al., 2000; Hughes et al., 2001; Townsend et al., 2003; Eguchi et al., 2004), as well as comparative malocclusion studies of aboriginal/rural vs. modernized/urban populations (e.g., (McCann et al., 1966; Niswander, 1967; Lavelle, 1968; Corruccini and Whitley, 1981)). Additionally, a large number of animal feeding experiments have demonstrated that compared to control groups, animals fed soft foods tend to develop smaller corpus bones, dental arches, palates, and mandibular rami, and are significantly more prone to developing malocclusions (e.g. (Watt and Williams, 1951; Beecher and Corruccini, 1981; Corruccini and Beecher, 1982; Beecher et al., 1983; Corruccini and Beecher, 1984; Ciochon et al., 1997; Tokimasa et al., 2000; Maki et al., 2002; Lieberman et al., 2004; Larsson et al., 2005)).

The objective of this chapter is to examine the effect of food processing on the integration of occlusion. It is reasonable to hypothesize that the overall size/fit of the teeth and jaws are integrated by the masticatory forces generated during development. As an animal grows, the masticatory morphology responds to the amount of force generated. Should a developing animal consume foods that require relatively low chew forces, the covariation among the teeth and jaws is reduced and occlusion is compromised. In most cases, however, the poor fit among the teeth and jaws is a transient condition because malocclusion affects masticatory performance by decreasing chewing efficiency and increasing relative masticatory force. The production of higher forces act to promote covariation among the masticatory structures and a morphologically integrated *adult* cranio-dental complex results. In other words, the growth of the masticatory system is integrated at least in part because how the teeth and jaws fit together affects function. If the relationship between morphological integration and masticatory

performance is weakened (such as when chewing processed foods), then the feedback loop is disrupted and the adult teeth are maloccluded.

Two related studies will be presented. The first couples the masticatory performance data collected in Chapter 3 with subject occlusal scores. Although the data collected in the previous chapter cannot test the details of the specific mechanisms by which the masticatory system remains/becomes integrated under different conditions, they do provide an opportunity to test whether the strength of morphological integration (based on objective scores of the subject occlusion) affects function, and if this relationship changes depending on whether the food is processed or raw. This is a novel approach to studying cranio-dental integration and uses experimental methods to directly test whether or not food processing has relaxed the functional integration of the jaw. The general hypothesis to be tested is that the loss of occlusal integration (represented by higher scores of malocclusion) leads to reduced masticatory performance (i.e. higher number of chews, force per chew, and total masticatory force, and lower comminution efficiency), and that the relationship between occlusal integration and mastication is lower when consuming processed versus raw foods. If the later proves true, then the 'disuse' hypothesis of reduced masticatory integration in modern populations consuming soft, highly processed foods is supported.

The second study presented in this chapter tests if the teeth and jaws are integrated via epigenetic processes involving masticatory strain. It is well understood that bone responds to loading in many ways (for review, see Pearson and Lieberman (2004)). Through complex mechanisms bones mechanically sense strain, causing the activity of bone cells (osteoblasts, osteoclasts and osteocytes) to be modulated via paracrine/autocrine regulation of a number of growth factors (e.g. (Baylink et al., 1993; Mikuni-Takagaki et al., 1996; Mehrotra et al., 2004; Janssens et al., 2005)). Thus, consuming a harder, more mechanically demanding diet will induce high bone strains that will cause growth factors

to promote bone growth and remodeling, especially in young animals that have not yet reached skeletal maturity.

Although the effects of force on bone development are well documented, it has long been assumed that these same forces do not affect the development of the dentition. This, however, may not be true. In humans and other slow-growing diphyodont mammals the permanent dentition develops over an extended period of time. Adult teeth form in a crypt within the alveolar bone of the jaw, thus mastication occurs on deciduous dentition as the adult teeth form. Lieberman (2011) hypothesized that this creates a dynamic environment whereby the permanent teeth are developing within a jaw that is being loaded by masticatory forces, leaving open the possibility that strain in the jaw can affect growth of the teeth as well as bone. For example, numerous *in vivo* and *in vitro* studies have demonstrated that insulin-like growth factor-1, which is upregulated when bones and sutures are strained (e.g. (Lean et al., 1995; Mikuni-Takagaki et al., 1996; Kumei et al., 2002; Hirukawa et al., 2005), increases both bone cell proliferation (e.g. (Tokimasa et al., 2003; Sakata et al., 2004)) and dental cell activity (e.g. (Young et al., 1995; Caton et al., 2005; Fujiwara et al., 2005)). It is therefore reasonable to predict that increases in masticatory force production would upregulate IGF-1 and subsequently result in a larger jaw and teeth.

A number of lines of evidence suggest that masticatory forces do in fact have some influence on the dentition. First, the narrow-sense heritability of molar occlusal dimensions (maximum mesio-distal and bucco-lingual widths) is approximately 0.60 (Townsend et al., 2009), which means that a large portion of molar size variance is attributable to non-genetic factors, such as the forces generated during chewing. Second, people with cleft lip/palate syndromes have smaller permanent, but not deciduous dentitions than non-cleft controls (Harris, 2002), presumably because the development of the permanent teeth is subject to more post-natal environmental factors (mastication, swallowing, etc.) than the earlier forming deciduous teeth. Finally, Brace (1991) notes a secular decrease in crown size

(approximately 1% per 1000 years) throughout the Holocene, which may correspond to the increasing use of food processing technologies that significantly reduce masticatory forces (e.g. the use of clay pots to boil foods, etc.).

Most research focuses on the crown portion of the tooth, however, it is the dental roots that may be most responsive to masticatory loading because they are still developing even after the tooth is in full occlusion and bearing the full masticatory load. While very little research has been done in this area, there are some indications that dietary loading does indeed affect the development of root. First, in one of the only studies to compare root dimensions directly to dietary force production, Spencer (2003) found that distantly related primate taxa who consumed tough seeds had larger root surface areas than closely related taxa with less resistant diets. A second line of evidence linking root development and masticatory force comes from Tonge and McCance (1973) who compared the jaw and dental development of pigs fed an *ad libitum* diet to an experimental group who were calorically restricted for the first 12 months of their life. The experimental pigs had significantly reduced body mass (~5.5 kg versus 180.0 kg for normal-fed pigs), delayed dental development, increased incidents of malocclusion, and smaller tooth roots, particularly in the molar region. Although nutritional stress undoubtedly played a large role in causing these morphological differences, masticatory loading was also greatly reduced in the calorically deprived group and might have further contributed to the differences.

The second experiment described in this chapter is a preliminary study that uses two groups of pigs fed hard and soft foods to test the hypothesis that masticatory forces integrate occlusion by modulating the growth of both the jaws and dentition. Particular emphasis is placed on the corpus of the jaws and the dentine tissue of the tooth, which makes up the bulk of the tooth root and is also found deep to the enamel in the crown. Although this study will not examine the particular molecular

mechanisms that integrate the system, it does test if masticatory loading affects tooth and jaws size in a similar manner (i.e. they both get smaller, larger, or do not change), which will alter how they fit together and overall occlusal integration.

Experiment #1, Occlusion and Masticatory Performance

Methods

Data Collection (Occlusion).

Plaster dental casts were created for each subject who participated in the experiments described in Chapter 3. Occlusion was then scored from the dental molds using a standard ruler (accuracy, ± 1mm) and the peer assessment rating (PAR) index (Richmond et al., 1992), a well-described, common scoring metric used in orthodontic practices worldwide. The PAR index is composed of five main variables that are scored and then summed to create a single score of a person's occlusion. The higher the score, the more deviant the occlusion is from normal. Some of the variables described below are weighted to increase their overall effect on the total occlusal score. See Figure 4.1 for specific scoring details. Measurement precision was quantified by scoring one randomly chosen dental mold five times.

1) **Upper and Lower Anterior Segments.** The distance between the contact points of each incisor tooth was measured and scored. Scores for each tooth (upper and lower) were summed together to create one score for the anterior segment relationship.

2) **Right and Left Buccal Occlusion.** The buccal occlusion of the molars and premolars was scored in three planes; anterior alignment, presence/absence of an open bite (vertical alignment), and transverse alignment. Both right and left buccal occlusion was scored in these three planes and the results added together.

3) **Overjet.** The amount of incisor and canine overjet (anterior projection) or anterior crossbite (no anterior projection) was measured. The score from the highest scoring tooth was recorded and multiplied by six to calculate the total overjet score.

4) **Overbite/ Open Bite.** The amount of incisor and canine vertical space (open bite) or vertical overlap (overbite) between upper and lower teeth was measured. The score from the highest scoring tooth was recorded and multiplied by two to calculate the total overbite/ open bite score.

5) **Centerline.** The deviation of the upper dental arch center (contact points between the central incisors) relative to the lower dental arch center was scored and multiplied by four to calculate the total centerline score.

Data Collection (Tooth Size).

Occlusal surface area creates the stresses necessary to fracture food and may therefore be highly correlated with masticatory performance and confound the effects of occlusal score on performance. In order to assess the degree to which tooth size affects performance, the maximum bucco-lingual and mesio-lingual widths of the first molar were measured using digital calipers (accuracy, 0.01 mm) and the occlusal surface area of the tooth calculated as the product of the two measures (following (Wood, 1991)). Only the left teeth were measured, and for each subject the occlusal area of the upper and lower molars were averaged. (N.B., the first molar was measured because masticatory forces quantified in Chapter 3 were calculated from forces measured at this tooth.) Measurement precision was quantified by measuring the occlusal surface area of one randomly chosen first molar five times.

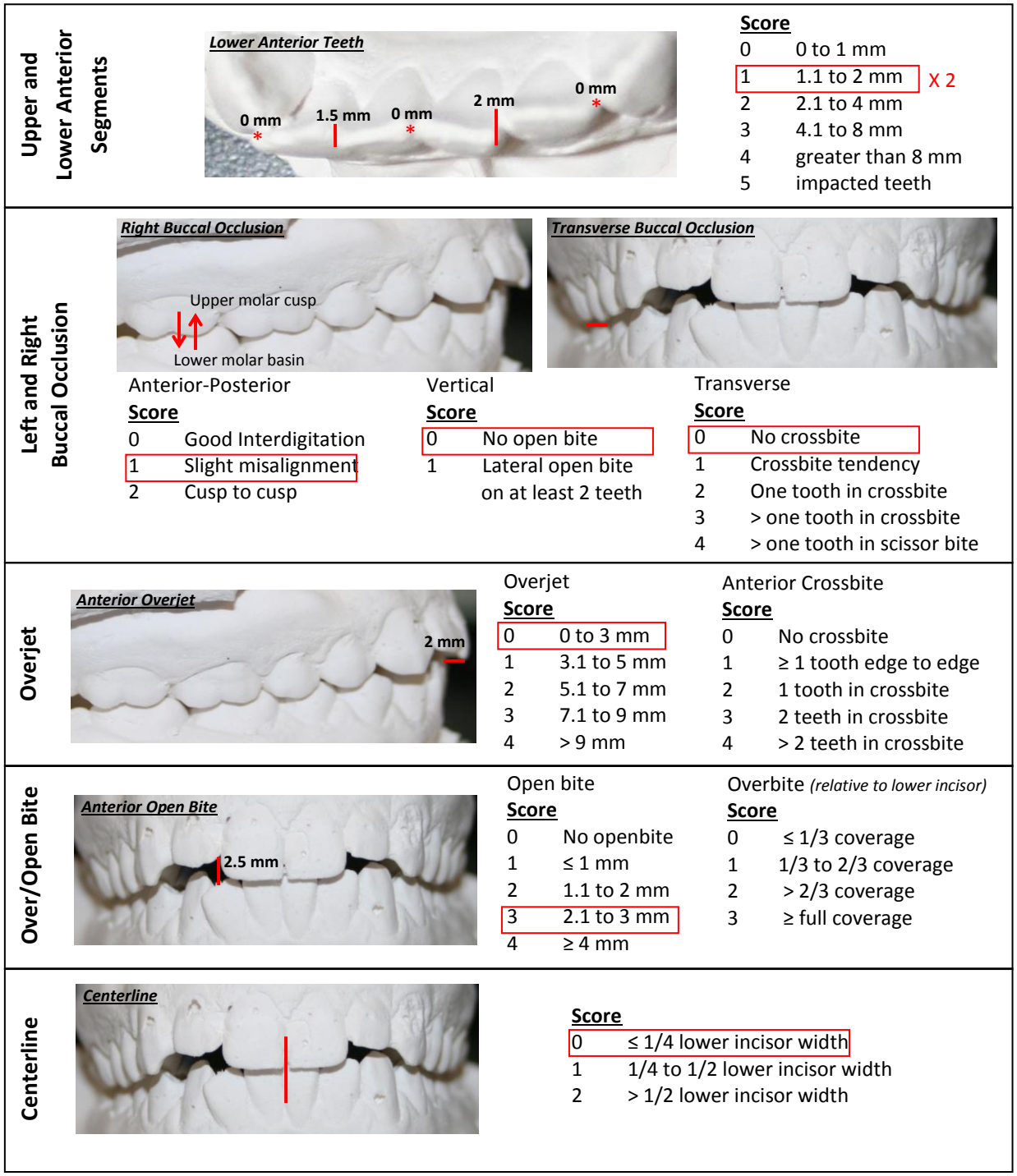

Figure 4.1. *The five major variables and scoring criteria for the peer assessment rating (PAR) index (Richmond et al., 1992). Scoring of a representative dental cast is shown. Upper (not shown) and lower anterior score = 2; Left (not shown) and right buccal occlusion score = 1; Overjet score = 0 X 6 (weighted) = 0; Over/Open bite score = 3 X 2 (weighted) = 6; Centerline score = 0 X 4 (weighted).* ***Total score = 9****.*

Analyses.

Meat and tuber samples were not standardized to the same size and since size influences masticatory performance, these foods were analyzed separately. Analyzed performance variables included number of chews, force per chew, total masticatory force and size of the largest comminuted particle. Tuber-specific comminution performance variables, number and total surface area of comminuted particles at 'swallow', as well as the average and median (50^{th} percentile) particle size, were also analyzed. For each subject, average masticatory performance was calculated in three ways, 1) average of *all* tubers or meat (raw and processed foods averaged), 2) average of all *raw* tubers or meat, and 3) average of all *processed* tubers or meat. These variables were then linear regressed against subjects' PAR occlusal scores and first molar occlusal areas. Multiple regression analyses were also performed to assess the combined explanatory power of occlusal score and first molar occlusal area for masticatory performance. Significance was set to ≤ 0.05. The Akaike Second Order Information Criterion (AICc) was calculated for each significant multiple regression and the linear regressions of the corresponding explanatory variables. If AICc was reduced by ≤ 2, the multiple regression was deemed a better predictor of masticatory performance than occlusion or first molar occlusal area alone. All analyses were performed in Excel (Microsoft 2007).

Results

Variation in intra-subject masticatory performance was quite low, even when raw and processed samples are pooled together (experiment #1 - tuber masticatory force, Table 4.1; experiment #2 - tuber comminution, Table 4.2; experiment #3 - meat masticatory force and comminution, Table 4.3). Subject PAR occlusal scores (range 0 – 29; average 9.3±9.2) and first molar occlusal areas (range 67.8 - 94.6 mm^2; average 80.5±7.9 mm^2) did not differ among the three different masticatory

experiments presented in Chapter 3 ($p > 0.05$, Wilcoxon signed rank test). Measurement precision was high; PAR score did not differ among the five repeats and the standard deviation of first molar occlusal area was 3.3 mm^2, which was approximately 5% of the repeated measurement average (67.9 mm^2).

No significant linear regressions resulted from using occlusal score as the single explanatory variable for subject masticatory performance (Table 4.4). In contrast, first molar occlusal area was negatively associated with both the number of chews (Figure 4.2) and the total masticatory force (Figure 4.3) used to consume all tubers, and explained 28% ($p=0.05$) and 43% ($p=0.01$) of the data variance, respectively. For both of these performance parameters, the explanatory power of the regression remained essentially the same when raw and processed tubers were analyzed separately (raw tuber chew number $r^2=0.27$, $p=0.05$; processed tuber chew number $r^2=0.28$, $p=0.05$) (raw tuber total masticatory force $r^2 = 0.44$, $p<0.01$; processed tuber total masticatory force $r^2=0.43$, $p=0.01$). Combining PAR occlusal scores with first molar occlusal area nearly doubled the explanatory power of the regression for number of chews used to consume tubers ($r^2=0.49$, $p=0.02$; AICc difference = 2.75), but had no effect on the explanatory power for total masticatory force ($r^2=0.47$, $p=0.03$; AICc difference = 1.18). The multiple regression of raw tubers explained 45% of the variance in chew number ($p=0.04$), slightly less than the 50% variance explained for processed tubers ($p=0.02$). Total masticatory force variance was explained equally well when raw tubers ($r^2=0.47$; $p=0.03$) and processed tubers ($r^2=0.46$; $p=0.03$) were analyzed separately.

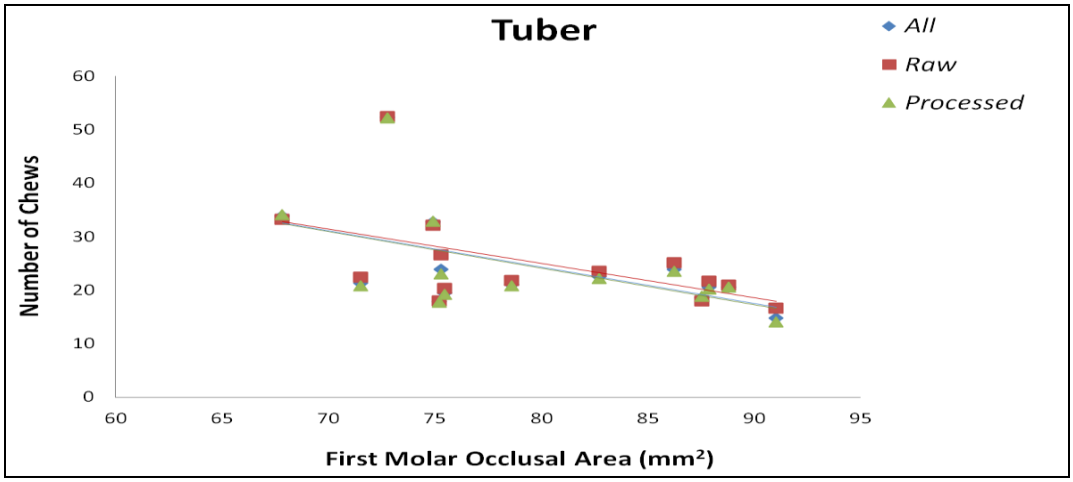

Figure 4.2. *The number of chews used to consume tuber samples regressed on subject first molar occlusal area (mm^2). Blue diamond = all meat samples (r^2=0.28); Red square = raw meat samples (r^2=0.27); Green triangle = processed meat samples (r^2=0.28). All regressions were significant (p≤0.05).*

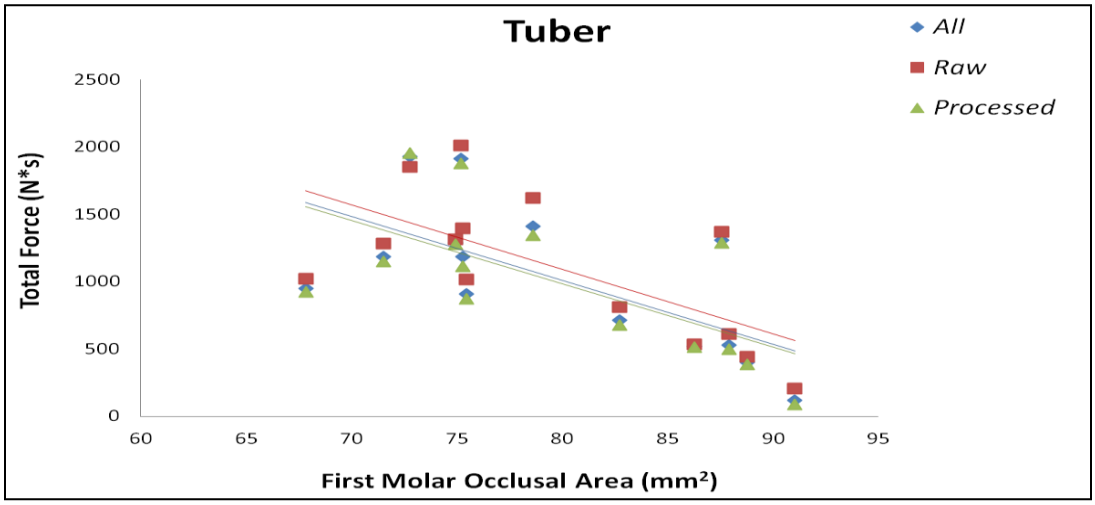

Figure 4.3. *Total masticatory force (N*s) used to consume tuber samples regressed on subject first molar occlusal area (mm^2). Blue diamond = all meat samples (r^2=0.43); Red square = raw meat samples (r^2=0.44); Green triangle = processed meat samples (r^2=0.43). All regressions were significant (p≤0.05).*

In addition to tuber masticatory force performance, first molar occlusal area was also negatively associated with raw tuber total comminuted particle area, and explained 59% of the variance in total particle area ($p<0.01$) (Figure 4.4). It should be noted, however, that this significant relationship was driven primarily by two subjects. Combining PAR scores and first molar occlusal area into a multiple regression did not increase explanatory power over that of the latter explanatory variable alone ($r^2=0.69$, $p=0.02$; ; AICc difference = 0.67). These same regressions were not significant for processed tubers or when all tubers (raw and processed) were analyzed together. Additionally, no other measures of tuber comminution performance were significantly correlated with subject occlusal score or molar occlusal size.

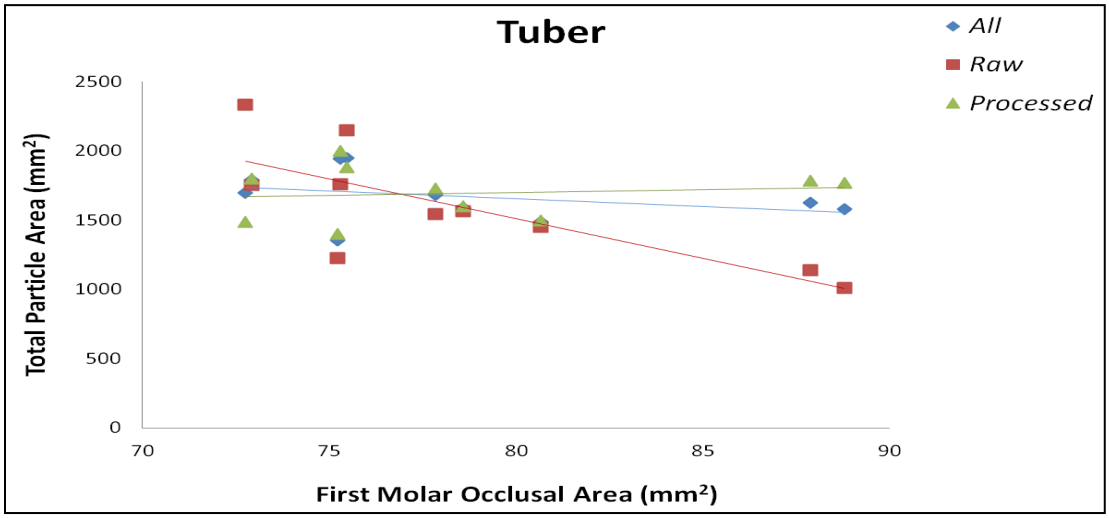

Figure 4.4. *Total area (mm^2) of comminuted tuber particles at 'swallow' regressed on subject first molar occlusal area (mm^2). Blue diamond = all meat samples; Red square = raw meat samples; Green triangle = processed meat samples. Only the raw tuber regression was significant ($p\leq0.05$) ($r^2=0.59$).*

In contrast to the tuber data, first molar occlusal area was significantly associated with the comminution performance resulting from consuming both raw and processed meat. First molar occlusal

area explained 67% ($p<0.01$) of the overall variance in size of the largest comminuted meat particle at 'swallow' (Figure 4.5). When raw meat was analyzed separately, the explained variance increased to 74% ($p<0.01$). In comparison, the regression for processed meat samples explained a much lower 58% ($p<0.01$) of particle size variance. Combining occlusal scores with first molar occlusal area did not change the explanatory power of the regressions (all meat $r^2=0.69$, $p=0.02$; ; AICc difference = 1.13) (raw meat $r^2=0.76$, $p<0.01$; AICc difference = 1.44) (processed meat $r^2=0.62$, $p=0.03$; AICc difference = 1.13).

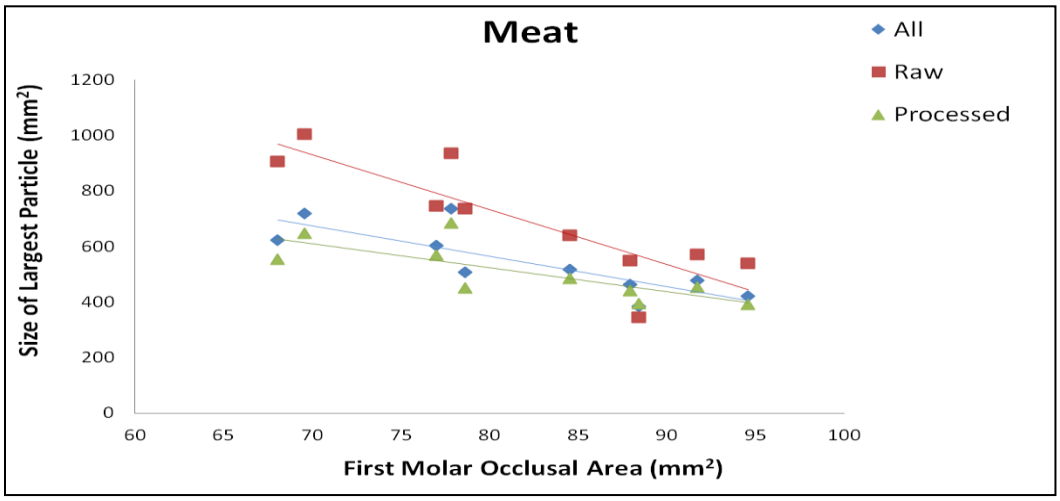

Figure 4.5. *Size (mm^2) of the largest comminuted meat particle at 'swallow' regressed on subject first molar occlusal area (mm^2). Blue diamond = all meat samples ($r^2=0.67$); Red square = raw meat samples ($r^2=0.74$); Green triangle = processed meat samples ($r^2=0.58$). All regressions were significant ($p\leq0.05$).*

Although first molar occlusal area alone did not significantly explain the variance in number of chews used to consume meat samples, when it was combined into a multiple regression with PAR occlusal scores, the regression explained 77% of the chew number variance for all meat samples ($p<0.01$; AICc difference = 9.65), and 69% and 74% of chew number variance for raw ($p=0.02$; AICc difference = 8.24) and processed ($p<0.01$; AICc difference = 8.45) meat samples, respectively.

Table 4.1. Subject dental morphology (PAR occlusal score and first molar occlusal area) and average chew number and masticatory force used to consume tubers. [a]

	PAR Score	First Molar Area (mm^2)	Chew Number	Force (N*s)	
				Per Chew	*Total*
Subject 1	8	75.5	19.5 (2.5)	46.2 (5.4)	908.7 (187.7)
Subject 2	29	71.5	21.3 (3.9)	55.3 (6.0)	1182.7 (266.0)
Subject 3	8	67.8	33.8 (5.2)	28.3 (4.5)	947.1 (160.1)
Subject 4	0	72.8	52.3 (5.6)	36.7 (3.6)	1927.9 (356.6)
Subject 5	13	82.7	22.5 (2.4)	31.6 (6.4)	711.4 (152.7)
Subject 6	15	75.2	17.8 (1.2)	106.9 (11.5)	1910.3 (256.6)
Subject 7	11	75.3	23.9 (5.1)	49.2 (6.1)	1185.1 (295.0)
Subject 8	0	91.0	14.7 (2.2)	7.9 (4.5)	119.0 (72.5)
Subject 9	4	88.8	20.6 (2.9)	19.2 (2.9)	399.3 (89.0)
Subject 10	0	74.9	32.7 (7.2)	38.3 (6.2)	1286.5 (402.4)
Subject 11	2	78.6	21.1 (2.7)	66.8 (7.4)	1412.6 (265.8)
Subject 12	14	86.2	23.9 (3.4)	21.7 (3.1)	519.7 (113.6)
Subject 13	0	87.5	18.6 (2.9)	69.9 (8.3)	1307.2 (278.6)
Subject 14	0	87.9	20.5 (2.2)	25.3 (6.6)	527.2 (159.3)

[a] *See Chapter 3 for details of masticatory performance experiments. Data for raw and processed tubers pooled for each subject. One standard deviation in parentheses.*

Table 4.2. Subject dental morphology (PAR occlusal score and first molar occlusal area) and measures of beet comminution at 'swallow'. [a]

	PAR Score	First Molar Area (mm^2)	Particle Number	Particle Size (mm^2)			
				Total	*Average*	*50th Percentile*	*Largest*
Subject 1	8	75.5	1668.7 (679.3)	1949.5 (175.6)	1.3 (0.4)	0.15 (0.12)	42.7 (16.0)
Subject 2	8	72.9	903.5 (232.9)	1787.7 (182.1)	2.1 (0.6)	0.33 (0.04)	42.5 (17.3)
Subject 3	2	78.6	1336.3 (259.8)	1591.9 (193.6)	1.2 (0.3)	0.28 (0.04)	25.7 (4.0)
Subject 4	15	75.2	1053.5 (411.5)	1355.8 (450.8)	1.3 (0.4)	0.31 (0.05)	23.9 (9.7)
Subject 5	4	88.8	1185.3 (378.1)	1578.5 (618.6)	1.3 (0.4)	0.25 (0.03)	38.0 (22.3)
Subject 6	0	72.8	1723.5 (864.0)	1697.8 (662.8)	1.1 (0.3)	0.28 (0.06)	27.8 (17.6)
Subject 7	0	87.9	2204.5 (457.9)	1623.8 (751.2)	0.7 (0.2)	0.19 (0.07)	18.3 (5.7)
Subject 8	26	77.8	801.8 (111.4)	1683.8 (269.1)	2.1 (0.5)	0.33 (0.09)	40.3 (19.0)
Subject 9	2	80.7	774.8 (156.0)	1485.0 (184.4)	2.0 (0.6)	0.24 (0.05)	65.0 (29.6)
Subject 10	11	75.3	904.5 (210.8)	1942.0 (299.2)	2.2 (0.5)	0.33 (0.12)	77.9 (12.9)

[a] *See Chapter 3 for details of masticatory performance experiments. Data for raw and processed beets pooled for each subject. One standard deviation in parentheses.*

Table 4.3. Subject dental morphology (PAR occlusal score and first molar occlusal area) and average chew number and masticatory force used to consume meat, as well as size of the largest comminuted particle at 'swallow'. [a]

	PAR Score	First Molar Area (mm^2)	Size of Largest Particle (mm^2)	Chew Number	Force (N*s)	
					Per Chew	*Total*
Subject 1	2	91.7	476.9 (208.7)	27.7 (7.6)	8.2 (6.8)	264.9 (258.3)
Subject 2	2	78.6	506.9 (234.7)	29.1 (5.9)	59.5 (18.3)	1781.0 (711.9)
Subject 3	0	87.9	462.2 (140.5)	44.9 (9.1)	73.9 (11.9)	3383.8 (1236.0)
Subject 4	19	88.4	384.3 (189.3)	59.8 (7.4)	30.4 (5.9)	1818.7 (424.6)
Subject 5	8	84.5	516.3 (143.6)	23.8 (5.8)	61.1 (15.2)	1429.7 (364.4)
Subject 6	21	94.6	421.2 (200.7)	80.7 (13.7)	35.0 (4.4)	2842.4 (681.7)
Subject 7	1	77.0	603.2 (238.9)	15.7 (5.3)	49.4 (11.9)	827.0 (435.8)
Subject 8	23	68.0	623.7 (270.1)	33.3 (15.6)	-	-
Subject 9	26	77.8	735.1 (304.6)	54.3 (14.3)	-	-
Subject 10	29	60.4	718.2 (270.6)	31.1 (10.0)	40.7 (8.3)	1307.2 (527.0)

[a] *See Chapter 3 for details of masticatory performance experiments. Data for raw and processed meat pooled for each subject. One standard deviation in parentheses.*

Table 4.4. Regression of masticatory performance parameters on subjects' PAR occlusal scores and first molar occlusal area. [ab]

	TUBERS			MEAT		
	Number of Chews			**Number of Chews**		
	All	Raw	Processed	All	Raw	Processed
PAR Score	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
First Molar Area	**0.28 (-)**	**0.27 (-)**	**0.28 (-)**	*n.s.*	*n.s.*	*n.s.*
Multiple Regression	**0.49**	**0.46**	**0.50**	**0.77**	**0.69**	**0.74**
	Force per Chew *(N*s)*			**Force per Chew** *(N*s)*		
	All	Raw	Processed	All	Raw	Processed
PAR Score	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
First Molar Area	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Multiple Regression	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
	Total Force *(N*s)*			**Total Force** *(N*s)*		
	All	Raw	Processed	All	Raw	Processed
PAR Score	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
First Molar Area	**0.43 (-)**	**0.44 (-)**	**0.43 (-)**	*n.s.*	*n.s.*	*n.s.*
Multiple Regression	**0.47**	**0.47**	**0.46**	*n.s.*	*n.s.*	*n.s.*
	Size Largest Particle *(mm^2)*			**Size Largest Particle** *(mm^2)*		
	All	Raw	Processed	All	Raw	Processed
PAR Score	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
First Molar Area	*n.s.*	*n.s.*	*n.s.*	**0.67 (-)**	**0.74 (-)**	**0.58 (-)**
Multiple Regression	*n.s.*	*n.s.*	*n.s.*	**0.69**	**0.76**	**0.62**
	TUBERS ONLY					
	Number of Particles			**Total Particle Area** *(mm^2)*		
	All	Raw	Processed	All	Raw	Processed
PAR Score	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
First Molar Area	*n.s.*	*n.s.*	*n.s.*	*n.s.*	**0.59 (-)**	*n.s.*
Multiple Regression	*n.s.*	*n.s.*	*n.s.*	*n.s.*	**0.69**	*n.s.*
	Average Particle Area *(mm^2)*			**Median Particle Area** *(mm^2)*		
	All	Raw	Processed	All	Raw	Processed
PAR Score	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
First Molar Area	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
Multiple Regression	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

[a] *See Chapter 3 for details of masticatory performance experiments.*

[b] *Only significant r^2 values shown, $p \leq 0.05$. r^2 values significant at the $p \leq 0.01$ level are underlined.*

n.s. = not significant. Positive (+) or negative (-) associations indicated in parentheses. For both tubers and meat, multiple regressions combining PAR score and first molar occlusal area better explained the variance in number of chews than occlusal area alone (AICc difference ≤ 2).

Experiment #2, Jaw and Dental Development

Methods

Experimental Animal.

Standard Yorkshire pigs (*Sus scrofa*) were chosen as a model organism for human jaw and dental development. Pigs are a useful model for dental studies because of their similarity to humans; they are diphyodont (possessing two sets of dentition), have bunodont molars, and most importantly, their dental growth trajectory is extended, which causes them to masticate with their deciduous dentition while the permanent teeth are still forming in the tooth crypt (Tonge and McCance, 1973; Hillson, 2005). In particular, this latter feature is key to studying how masticatory parameters affect the development of the permanent dentition.

Experimental Protocol.

Eight, six-week old male pigs were randomly split into two groups (n=4) and were fed *ad libitum* diets composed of hard foods (standard, hard pig chow and dried corn kernels) or isocaloric soft foods (water-softened pig chow and corn flour). In order to track dental development, the pigs were administered 30 mg/kg of calcein, a florescent mineral label, at the start of the experiment and every two weeks thereafter. After 12 weeks on the experimental diet the animals were sacrificed and their skulls cleaned.

Data Collection.

All data was collected from the right side of the skull. Maximum maxillary and mandibular corpus height and width were measured at the mesio-distal midpoint of the fourth deciduous premolar using standard calipers (accuracy, 0.01 mm). The first and second molars were then removed from the

jaws. The first molar was fully erupted and the experimental period captured root, but not crown formation. In contrast, the second molar was an incomplete dental crown that had to be extracted from the dental crypt. Approximately half of the second molars lacked the first florescent label, indicating that the experiment captured crown initiation. Because of its early stage of development, the enamel of the second molar was poorly mineralized and damaged by the cleaning and extraction protocols. Therefore, only the crown dentine was available for study.

A Buehler IsoMet™ saw was used to make a bucco-lingual cut though the mesial cusps of the teeth (paracone/protocone and protoconid/metaconid of the upper and lower teeth, respectively), bisecting the lingual apical opening of the roots in the first molar, and the inferior margin of the patent crown in the second molar. For the first molars, two sections were created from a single bisected cusp. Each dental piece was mounted, cut side down, onto a standard slide using epoxy and trimmed with the saw to create an approximately 800 micron section. These sections were then ground on a Hillquist™ thin section grinder using successively smaller grit and hand polished to reach a final thickness of approximately 150 microns. The second molars were similarly processed, however only one section was made per tooth because the small size of the cusps meant that one of the cut portions was always off center and did not include the very tip of the cusp.

A scaled image of each dental section was captured under florescence using an Olympus™ BX51 microscope with a Qimaging™ camera. Each section was photographed at 20x magnification (for measurement of dentine apposition), 4x (for measurement of root extension in the first two experimental weeks), and 1.25x (for measurement of root extension during the remainder of the experimental period). Measurements (described below) were taken on the lateral aspect of each lingual cusp using MicroSuite™ Imaging software.

Dentine Apposition. (Figure 4.6) To ensure homologous measurements within each tooth type, apposition was quantified at the dental cervix in the first molar and at 45% dentine horn height in the second molar. Measuring in these dental regions limited data collection to apposition that occurred between experimental weeks 4 and 10. The amount of dentine secreted during this six-week period was determined by measuring the length of a single dentine tubule between the florescent labels coinciding with experimental week 4 and 10. The lengths of 5 tubules per tooth were measured and the results averaged.

Root Extension. (Figure 4.6) The amount of root extension that occurred from the start of the experiment to experimental week 10 was quantified for the first molar. This was done by measuring the distance between the inferior margin of the label corresponding to week 0 and week 10, following the lateral margin of the root.

Analysis.

When two sections were available for measurement (as in the first molar), the smallest and therefore most conservative duplicate measurement was used in the analyses. Statistical differences between the dentine apposition and root extension of the hard and soft food groups were tested by re-sampling the data 10,000 times. All calculations were performed in Excel with the Poptools add-in (Microsoft 2007) and significance was set to $p \leq 0.05$.

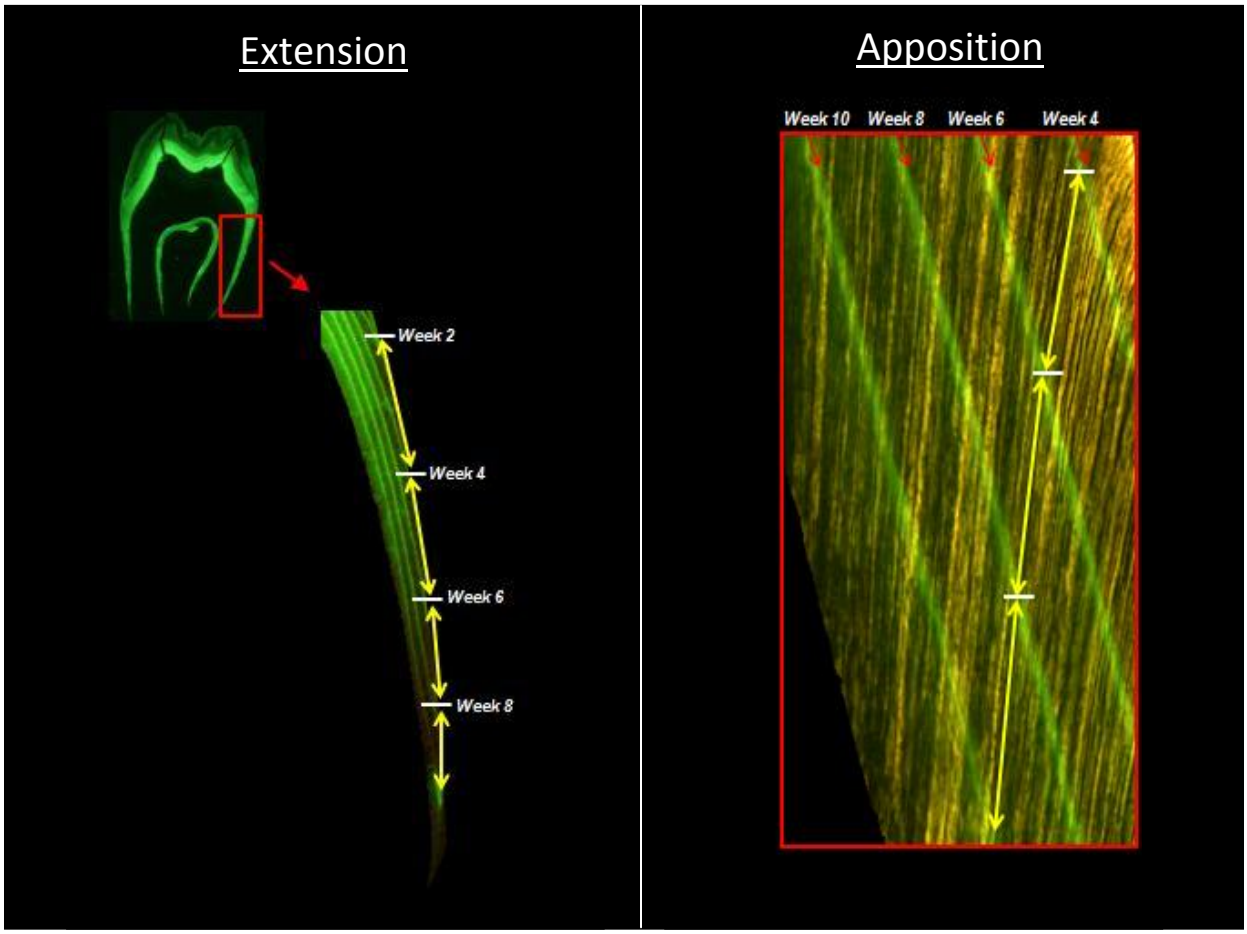

Figure 4.6. *Bucco-lingual section of the mesial cusps of the first molar, viewed under florescent light. Green lines are florescent labels created by calcein, a florescent marker that binds to the dentine deposited with ~24 hours of each injection.* ***LEFT****: Root extension was measured from the start of the experiment (label not shown) to experimental week 10, following the lateral margin of the root. Yellow arrows represent the distance that the root extended every two weeks.* ***RIGHT****: Dentin apposition was measured from experimental week 4 through 10. The amount of dentine secreted during this six-week period was determined by measuring the length of a single dentine tubule between the florescent labels. This distance is represented by yellow arrows in the image above.*

RESULTS

Diet affected the growth of the mandibular and maxillary corpora (Table 4.5). On average, pigs consuming a softer diet had a 9% shorter maxillary corpus (Soft Diet 47.14±1.01mm; Hard Diet 51.64±1.24mm; p = 0.01) and 8% shorter mandibular corpus (Soft Diet 32.27±1.90mm; Hard Diet 35.28±1.67mm; p = 0.04) than those fed hard foods. While width of the corpus bones did not differ significantly between the dietary groups (Maxilla - Soft Diet 14.58±1.26mm; Hard Diet 14.36±0.40mm; p = 0.36) (Mandible - Soft Diet 17.06±1.45mm; Hard Diet 18.43±0.29mm; p = 0.06), there was a trend whereby soft-fed animals tended to have 7% more narrow mandibular corpora.

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Table 4.5. Average height and width of the maxillary and mandibular corpus. [a]

	Maxillary Corpus *(mm)*		Mandibular Corpus *(mm)*	
	Width	Height	Width	Height
Hard Diet *(n=4)*	14.58 *(14.25 - 15.15)*	51.64 *(50.25 - 53.25)*	18.43 *(18.14 - 18.84)*	35.28 *(33.08 - 37.12)*
Soft Diet *(n=4)*	14.36 *(13.10 - 15.76)*	47.14 *(46.12 - 48.43)*	17.06 *(15.35 - 18.58)*	32.27 *(30.29 - 33.94)*
p-value [b]	*0.36*	***0.01***	*0.06*	***0.04***

[a] *Measurements were taken at the level of the first molar. Range of measurements in parentheses.*

[b] *Statistical differences between pigs fed hard and soft food groups were tested by re-sampling the data 10,000 times. P-values ≤ 0.05 are highlighted.*

Dental development was also effected by dietary loading (Table 4.6). Although diet had no effect on dentine apposition in the root of the first molar (Upper First Molar - Soft Diet 0.53±0.02mm; Hard Diet 0.57±0.05mm; p = 0.12) (Lower First Molar - Soft Diet 0.58±0.05mm; Hard Diet 0.59±0.01mm; p = 0.34), the animals who consumed a soft food diet did have 9% less dentine apposition in the crown of the lower second molar (Soft Diet 0.44±0.02mm; Hard Diet 0.48±0.03mm; p = 0.04), and tended to have 16% less dentine apposition in the crown of the upper second molar (Soft Diet 0.58±0.09mm; Hard

Diet 0.69±0.10mm; p = 0.09), although the latter effect did not reach statistical significance. Additionally, while root extension of the upper first molar did not differ between the dietary groups (Soft Diet 9.49±0.35mm; Hard Diet 9.26±0.26mm; p = 0.82), there was a trend whereby root extension of the lower first molar tended to be 6% less in the pigs fed soft foods (Soft Diet 11.29±0.57mm; Hard Diet 11.95±0.67mm; p = 0.08).

Table 4.6. Average dentine apposition and extension. [a]

	Dentine Apposition *(mm)*				Root Extension *(mm)*	
	First Molar		Second Molar		First Molar	
	Upper	Lower	Upper	Lower	Upper	Lower
Hard Diet *(n=4)*	0.57 (0.52 - 0.62)	0.59 (0.57 - 0.60)	0.69 (0.56 - 0.79)	0.48 (0.45 - 0.52)	9.26 (9.01 - 9.64)	11.95 (11.15 - 12.75)
Soft Diet *(n=4)*	0.53 (0.50 - 0.55)	0.58 (0.53 - 0.64)	0.58 (0.51 - 0.70)	0.44 (0.42 - 0.46)	9.49 (9.10 - 9.78)	11.29 (10.75 - 12.00)
p-value [b]	*0.12*	*0.34*	*0.09*	***0.04***	*0.82*	*0.08*

[a] *Dentine apposition was measured between the florescent labels corresponding to experimental weeks 4 and 10. Root extension was measured from the first florescent label (start of the experiment) to the last florescent label (experimental week 10). Range of measurements in parentheses.*

[b] *Statistical differences between pigs fed hard and soft food groups were tested by re-sampling the data 10,000 times. P-values ≤ 0.05 are highlighted.*

DISCUSSION

The results of these two preliminary studies suggest that masticatory force acts as an integrating factor in the development of the teeth and jaws, and that both size of the teeth as well occlusal integration affect masticatory performance. First, as predicted, pigs fed a soft food diet had both smaller jaws and less dentine growth than animals consuming harder foods. This supports the hypothesis that force influences both jaw and tooth development, and leaves open the possibility that masticatory forces coordinate the growth of these structures to promote proper occlusion. Interestingly, the effects of masticatory loading were not evenly distributed among the teeth. The only significant change in dentine apposition ($p<0.05$) occurred within the second molars, perhaps because these teeth were at a more immature state than the first molars during the experimental period; located deep within the loaded jaw, the second molar may have been particularly sensitive to masticatory strains.

The results also suggest that masticatory forces might have disproportionate effects on mandibular structures compared to those of the maxilla. While both mandibular and maxillary corpus height were significantly altered by masticatory forces, mandibular width also tended to be affected ($p = 0.06$). Additionally, dental development of the lower molars tended to have a more consistent response to reduced masticatory loading, as evidenced by lower p-values. This response difference between the upper and lower jaws highlights their contrasting developmental constraints. Compared to the maxilla, the mandible is much more autonomous in its development and is less integrated with the rest of the skull's growth and functions. This means that mandibular morphology may be more likely to respond to exogenous factors such as diet, as found by von Cramon-Taubadel (2011) who showed that human subsistence strategy was significantly correlated with the mandible, but not other masticatory (e.g., palate/maxilla) or non-masticatory (e.g. cranial vault and base) structures.

Although the results of this animal experiment tend to support the hypothesis that masticatory force integrates the teeth and jaws, future studies should include larger sample sizes in order to confirm

that trends in the data (i.e. $p > 0.05$, but less than $p < 0.10$) reflect real differences between the dietary groups. Additionally, the experiments should be performed over longer periods of time. This study only captured a short period of development, about 12 weeks, and although growth rates declined as a result of less masticatory loading, it is not known if the end result would be a less-integrated, smaller structure, or if growth would continue over a longer period with no change in the adult morphology. It is very unlikely, however, that the adult morphology would remain unaffected. For example, Larsson et al. (2005) fed pigs hard or soft foods for a long period of time (nearly two years), and the adult soft-fed pigs exhibited more dental rotations and malocclusions. Further evidence for a long term effect on cranio-dental morphology comes from Tonge and McCance (1973). As discussed preciously, these researchers reduced the caloric intake (and masticatory loading) of piglets for twelve months, resulting in slowed dental development and extreme overcrowding. Two years after the caloric restriction was lifted, however, the adult morphology remained compromised, which suggests that growth can be perturbed for only a limited time period before the effects become permanent.

Functional Integration

The results of the human study partially support the 'disuse' hypothesis that the jaws and teeth are functionally integrated and that food processing has led to the malocclusion epidemic in modern, urban human populations (e.g., (Corruccini, 1999)). Although occlusal integration by itself was not associated with changes in masticatory performance, when added to a multiple regression with tooth size it greatly increased the predictive power over that of the former alone for the number of chews used to consume both meat and tubers. This suggests that occlusal integration does indeed have some effect on masticatory performance, although it may be subtle and possibly secondary to the effect of dental size. Subjects with larger teeth and higher measures of occlusal integration (lower PAR scores) used fewer chews to consume both meat and tubers, and were able to comminute the foods more

efficiently; meat was swallowed at a more fractured state, and even though tubers were chewed less (and with less force), the resulting bolus was just as fractured as those created by subjects with smaller, more maloccluded teeth.

The association between cranio-dental morphology and masticatory performance may be especially important when consuming foods like meat, as our teeth are poorly adapted to shearing such extensible material (see Chapter 3). For meat, the combined effect of dental size and occlusal integration was particularly informative, explaining 77% of the variance in number of chews used and 69% of the variation in size of the largest swallowed particle. In contrast, tubers are readily fractured between human bunodont molars and as a result, relatively less variance (49% of chew number and 47% of total masticatory force) was explained by dental size and integration. Additionally, there was no relationship between morphological measures and how fractured the tuber bolus was at swallow. While using less chews and total masticatory force will reduce masticatory costs, as discussed numerous times throughout this dissertation, swallowing a more fractured bolus when consuming meat may have particularly significant effects on net energy gain by reducing the cost of digestion (Boback et al., 2007; Carmody, 2012).

Although occlusal integration affected masticatory performance, unlike what was hypothesized there was no decrease in the strength of the association between occlusion and masticatory performance when consuming raw versus processed foods. The only instance where masticatory morphology better predicted the consumption of raw compared to processed foods was total particle area of the swallowed tuber bolus. This result may not hold at larger sample sizes, however, as it was driven primarily by two subjects with particularly large teeth who also produced boluses with less total particle area than the other subjects. Further highlighting the fact that this result should be viewed with caution, none of the other five measures of tuber comminution were significantly associated with these morphological parameters.

Even though there was no clear difference between the amount of variance explained when chewing raw versus processed foods, the "disuse" hypothesis should not be rejected. Malocclusions are a relatively recent problem, and it is reasonable to predict that the performance differences between raw foods and those processed using Paleolithic techniques were not large enough to pick up a signal of functional integration, particularly at the low sample sizes available for this study. The processing methods used in this dissertation (slicing, tenderization and roasting) would likely have small effects on masticatory performance compared to changes caused by consuming extremely processed, modern foods.

There are at least three ways that future studies can improve on the current experimental design. The first is to design experiments to maximize masticatory performance differences between the treatment foods by having subjects chew foods that require extremely low and high masticatory effort. For example, subjects can chew volume-standardized samples of hard versus super processed foods, such as peanuts and peanut butter. Another simple approach to testing the 'disuse' hypothesis is to have subjects chew artificial test foods of known material properties, such as soft and hard gels or color changing gum/wax. Although these materials are not natural foods, they provide uniform samples that are ideal for controlled experiments and are routinely used in masticatory performance studies (e.g. (Iwase et al., 2006; Gambareli et al., 2007; Schimmel et al., 2007; Oueis, 2009; Speksnijder et al., 2009)). Of additional benefit, color changing gum/wax provides a quick assessment of comminution performance by analyzing the degree of color mixing that occurred during chewing.

Second, sample size should be increased in future studies. Sample size was extremely low in this experiment, ranging from eight to fourteen individuals depending on the masticatory performance variable. In contrast, most covariation integration studies require a sample size of at least thirty to forty to reach significance. A power analysis was run on the data and the results indicate that a sample size of thirty, which is at the lower range of sample sizes used in previous studies linking broad categories of

malocclusion to performance deficiencies (e.g., (Iwase et al., 2006; Toro et al., 2006)), would result in a significant relationship between occlusion and many masticatory performance variables collected in the dissertation chapter ($p \leq 0.05$; 80% power; Simple Interactive Statistical Analysis, www.quantitativeskills.com/sisa). All else being equal, if sample size was increased to thirty, lower occlusal integration would be significantly associated with the use of more chews to consume meat, and lower measures of tuber and meat comminution efficiency.

A final avenue for improvement is to use a different measurement of integration. A possible reason for non-significance in this experiment is that the PAR occlusal score may do a poor job of quantifying the occlusal features most relevant to processing. The main purpose of the PAR score is to assess the need for orthodontic treatment, particularly in young children. By necessity, it is designed to measure occlusion at any dental stage and in doing so it loses measurement precision. An alternative to PAR is to use a more comprehensive orthodontic scoring technique that treats each tooth individually, or to ignore standard, orthodontic measures of malocclusion altogether and either design a unique occlusal score based on features predicted to be most important to performance (i.e. interproximal distances and occlusal contact areas), or simply use standard integration techniques to quantify overall morphological integration of the dental arches.

CHAPTER 5. CONCLUSIONS

The studies presented in this dissertation highlight the many effects that the adoption of food processing techniques have had in altering hominin masticatory performance and morphology. The results of these experiments lead to six main conclusions.

Conclusion #1: ***Mechanical processing techniques significantly reduce masticatory effort and improve comminution efficiency.*** It should be noted, however, that the two mechanical processing methods studied, slicing and tenderization (pounding), had different effects on how subjects masticated meat and tubers. In meat, slicing reduced masticatory force by approximately 20-25% and greatly improved comminution efficiency (size of the largest particle in the 'swallowed' sliced meat bolus was almost half that of the raw meat), but mechanical tenderization increased force per chew approximately 20% and had no effect on comminution. In tubers, mechanical tenderization reduced both masticatory force (11%) and the number of chews (~4%) used to consume the food, while slicing produced no measured change in masticatory effort.

The different effects of mechanical processing on the mastication of meat and tubers highlight key material property differences between the two foods. Tubers are a 'force-limited' food that fractures readily in compression between the teeth. Using a replica Oldowan hammerstone to mechanically tenderize tubers causes damage to the internal structure of the food, significantly decreasing measured toughness and consequently lowering masticatory force production. Slicing the tubers, however, does not affect how it was masticated, probably because this form of processing does not alter the food's intrinsic material properties and because tubers are readily comminuted raw. The behavior of meat, on the other hand, is entirely different. Meat is a 'space-limited' food that is not easily fractured between the low-cusped molars typical of hominins, which are ideal for crushing and grinding hard and stiff foods, but are poorly adapted to shear tough, elastic foods like meat. We can therefore conclude that using early stone tools to slice meat into smaller particle sizes before ingestion would

have been a key adaptation to improve the ability of hominins to effectively comminute this food source.

Conclusion #2: ***Cooking (roasting) tubers further reduces masticatory effort relative to mechanical processing techniques.*** Roasting significantly decreased the toughness, fracture stress and stiffness of tubers. Consequently, roasted tubers required approximately 10% fewer chews than raw tubers, and almost 15% less force per chew and 20% less total masticatory force. This total masticatory force reduction was a further 10% less than the force used to consume tenderized tubers. The effects of roasting translates into significantly reductions of masticatory effort. For example, if a hominin chewed 1 kg of tubers a day, approximately 2,340 fewer chews and 146 kN*s less masticatory force would be required if they were roasted. Interestingly, however, although roasting reduced the masticatory effort (force) required to chew tubers, it also decreased comminution efficiency and a less fractured bolus was formed when subjects chewed a standard number of times (20 times) and also when they chewed until they felt they would typically swallow.

Conclusion #3: ***While cooking (roasting) increases the masticatory force necessary to consume meat, it greatly enhances the effectiveness of comminution.*** Roasting increased meat toughness and fracture stress, leading to an approximately 25% increase in masticatory force per chew when meat was roasted to a high internal temperature (70°C, medium-well). Roasting decreased meat stiffness and elastic energy loss, however, which significantly improved the subjects' ability to effectively comminute the meat, as evinced by the largest particle in the 'swallowed' roasted meat bolus being nearly half the size of raw meat. When meat was roasted to a slightly lower internal temperature (55°, medium-rare), the effect of cooking on comminution was not as great as cooking to a higher temperature (the largest particle in the roasted meat bolus was only 25% smaller than that of raw meat at 'swallow'). Reduced

improvement in comminution of less-cooked meat compared to more-cooked meat was balanced, however, by no change in masticatory force relative to raw meat.

Even though roasting meat tended to increase how much force was used per chew at high internal temperatures, when total daily masticatory force was calculated for hominins using the limited foods and processing types studied in this dissertation, the overall effect of cooking was a pronounced 20% reduction in total daily masticatory force. This reduction was relatively constant regardless of the amount of meat consumed, likely because any increase in force per chew when consuming cooked meat was mitigated by fewer chews needed overall per unit calorie (roasted meat is approximately 30% more calorically dense than raw meat, likely because of water loss).

Conclusion #4: ***Regardless of whether food is raw or processed, increasing the amount of meat in the diet decreases total masticatory force.*** Although the average masticatory force required to chew a gram of food was similar for tubers and meat (e.g., 544.5 N*s versus 537.5 N*s for raw tubers and meat, respectively), because meat is calorically dense, total masticatory force per calorie of meat is greatly reduced relative to tubers. The results of modeling the daily masticatory force of *Homo* in Chapter 3 suggest that shifting from a diet composed of a small amount of meat (5%) to one of intermediate amounts of meat (25%) would reduce total masticatory force production by approximately 10%.

It is important to highlight the fact that the studies presented here assessed only masticatory forces, and did not consider force production during ingestion, when the anterior dentition are used to prepare a bite-sized piece of food for subsequent oral processing. During ingestion, the food is either fractured from direct force of the incisors or gripped between the front teeth and an outside force (i.e. the hands) applied to create a tensile fracture (Lucas, 2004). Food processing techniques would have certainly improved ingestion performance and might have been particularly relevant for the

consumption of meat. A major difficulty associated with consuming meat is getting a small bite-sized piece from a carcass into the mouth: because raw meat is highly elastic and possesses crack-blunting properties, it is very difficult to tear into pieces. Using teeth to do this task would likely require a substantial amount of time and muscular force, as indicated by observations of chimpanzees consuming prey in the wild. Chimpanzees have been observed chewing on small (~4kg) animal carcasses for 4-11.5 hours (Goodall, 1986; Wrangham and Conklin-Brittain, 2003) and one possible contributing factor for this extreme effort may be the work needed to obtain mouth-sized portions that can then be chewed and swallowed. In this regard, the advent of stone tool-based food processing techniques would have significantly improved the ability of hominins to efficiently ingest meat. Stone tools were used extensively to butcher animals and probably cut the meat into small pieces prior to consumption (e.g., (Semaw et al., 2003; Dominguez-Rodrigo et al., 2005; Bunn, 2007; Pobiner et al., 2008)). Cooking would have also aided ingestion of meat; heat denatures and reduced the fracture strength of collagen and other connective tissues, which would make it much easier to separate meat from the rest of the carcass if an animal is cooked whole. Additionally, because cooking stiffens meat and reduces elastic energy loss, it would be much easier (i.e., require less work) to use the incisors to remove a small bite-sized piece of meat from a larger portion.

Conclusion #5: ***Because masticatory force production influences jaw and dental growth (preliminary conclusion), changes in food processing technology affected masticatory morphology and integration.*** The results of the experiment using swine described in Chapter 4 support the hypothesis that masticatory force helps coordinate the growth of the teeth and jaws. The effect of force on the resulting adult morphology remains unknown, however, and future studies (discussed below) are needed to test whether a more morphologically integrated system is generated from the production of higher masticatory forces (as indicated in previous animal experiments (e.g. (Beecher and Corruccini, 1981; Corruccini and Beecher, 1982; Beecher et al., 1983; Corruccini and Beecher, 1984; Ciochon et al.,

1997; Larsson et al., 2005), and whether the degree of integration affects function. Assuming that masticatory forces promote integration and proper function, these data suggest that the prescriptive use of chewing gum may be a useful method to prevent or correct occlusal problems in growing children. (I am currently testing this hypothesis in collaboration with a pediatric orthodontist.)

Conclusion #6: ***Postcanine size and masticatory integration (assessed through occlusal scores) are positively correlated with measures of masticatory efficiency.*** Subjects with smaller teeth used more chews and total masticatory force to consume tubers, and when eating meat, they swallowed a less fractured bolus. These results support findings by other studies which show that decreases in masticatory performance are associated with fewer contact points between teeth (e.g. (Owens et al., 2002; Buschang, 2006)). This suggests that there is a potential masticatory "cost" associated with reductions in hominin tooth size within the genus *Homo*, and supports the idea that diet itself did not select for smaller teeth in *Homo*, but that a less mechanically demanding diet made possible selection for these reductions for other reasons, most likely the need to fit teeth into shorter and smaller faces (Lieberman, 2011). For example, the data collected in this dissertation indicate that if hominins roasted tubers prior to consumption they would have generated approximately 15% less force per chew than if they consumed raw tubers. Such a reduction of masticatory force *per chew* could have then mitigated any relative increases of *total* masticatory force caused by decreased hominin postcanine tooth size.

Although there was a clear relationship between molar occlusal area and masticatory performance, the effect of masticatory integration (as measured by occlusal score) on performance was much less pronounced. Occlusal score alone was not a significant predictor of masticatory performance, and its effect was apparent only when combined into a multiple regression with first molar size; subjects with higher levels of malocclusion (i.e., less integrated occlusion) used more chews to consume both tubers and meat than those with better occlusion. Although more research is needed to confirm these

results, these preliminary data suggest that the masticatory system is indeed functionally integrated in a way that affects chewing performance. Contrary to prediction, however, the relationship between masticatory integration and performance was the same for both raw and processed foods. It is possible to interpret these data as failing to support the hypothesis of "disuse", which posits that the malocclusion epidemic experienced by modern human populations is a consequence of consuming soft, overly processed food that do not generate enough masticatory force to integrate the development of the jaws and teeth (see Corruccini (1999) for review). It is not surprising, however, that the experiment found no difference between the consumption of raw and processed foods. Compared to modern food processing techniques (e.g., microwaves, mixers, blenders, etc.), the simple Paleolithic processing methods that were tested likely had much less of an effect on masticatory performance. Thus, further research is needed to thoroughly test the hypothesis that consuming modern highly, processed foods has reduced the functional integration of the masticatory system.

Implications for Hominins

What we know about the evolution of hominin cranio-dental morphology combined with the results of the data presented in this dissertation, suggest a three-phase shift in the evolution of hominin mastication. The first transition was a reduction in peak force per chew from australopiths to *H. habilis*. Eng et al. (2013 (in review)) documented a grade shift in occlusal stress that occurred during the evolution of the genus *Homo*, driven largely by a reduced ability to produce high masticatory forces. One likely explanation for this grade shift is the adoption of food processing techniques. Although when cooking first evolved and became commonly practiced is unknown (see Chapter 1 for review), mechanical processing techniques were surely practiced by at least 2.6 mya (Semaw et al., 1997), only briefly predating the oldest securely dated fossils that are attributed to *Homo* at 2.3 mya (Kimbel et al., 1997). Regardless of the type of processing methods employed, it is reasonable to predict that hominins

would choose to processes the foods that were the most mechanically demanding and thus required the highest masticatory forces to consume. Consumption of these foods provides a limit for the amount of masticatory force and dental stress that individuals must have been able to generate. By processing these mechanically demanding foods, peak masticatory force production would decrease and selection would no longer have to act to maintain energetically costly masticatory structures such as large muscles and heavy, robust facial bones, and instead might permit selection on other adaptations such as reduced facial length. Although the data collected in Chapter 3 examined only two food types and three processing methods, the results suggest that adoption of cooking techniques, especially the use of fire to roast tubers, may best explain a grade shift in peak masticatory forces. Analysis of peak masticatory EMG voltage indicate that 14% less muscular recruitment is needed per chew when consuming roasted versus raw tubers. Because masticatory EMG is highly correlated with chew force (Proschel and Raum, 2001), this suggests that peak force per chew would significantly decrease as well. No other foods (i.e. meat) or processing types reduced peak masticatory EMG recruitment.

A second shift in hominin masticatory effort probably occurred during the transition from *H. habilis* to *H. erectus.* Although there is much body size variation within *H. erectus*, in general this transition involved an increase in body mass and perhaps an increase in daily foraging range, both of which require an increase in the absolute number of calories an individual must consume. If food type were held constant (which is unlikely), this caloric increase translates into the consumption of more food and thus more chews taken per day and more total masticatory force. The use of food processing techniques, however, as well as the addition of more meat and other animal-based foods sources (i.e. soft fat and bone marrow) to the diet would have mitigated these force increases. Additionally, the approximately 21% smaller teeth of *H. erectus* compared to *H. habilis* (McHenry and Coffing, 2000) suggest that selection had acted to decrease to postcanine size, perhaps to facilitate shorter, more

prognathic faces with higher effective mechanical advantage, which more efficiently produce masticatory forces and resist the resulting bite strains.

Finally, the transition from *H. erectus* to the smaller and more gracile masticatory complex of *H. sapiens* (Brace, 1967; Brace and Mahler, 1971; Wolpoff, 1973; McHenry, 1994; Lahr, 1996; Lieberman, 2011) indicates a substantial decrease in masticatory force. When assessing changes within *H. sapiens*, there is a pronounced secular trend of reduced face and tooth size that culminates in modern populations. This suggesting the development and perhaps increasing reliance on complex food processing techniques that started in the Middle Paleolithic (which is coincident with widespread evidence for hearth cooking (see (James, 1989) for review)), continued through the Upper Paleolithic, and reached its pinnacle in modern culinary techniques. For the first time in evolutionary history, one can now consume foods that require little to no masticatory force production because of food processors, blenders, microwaves, and other technologies.

Beyond changes to masticatory biomechanics, the adoption of food processing techniques likely had significant effects on the overall energetics and time allocation of hominins. Food processing undoubtedly increased the net energy gained from foods, which may have allowed selection for absolutely larger bodies, reduced sexual dimorphism, relatively larger brains and smaller intestines, and shorter inter-birth periods (Wrangham et al., 1999; Boback et al., 2007; Carmody and Wrangham, 2009; Wrangham, 2009; Carmody et al., 2011). Net energy is a function of the total calories that a food contains minus the cost of food acquisition, ingestion, mastication, digestion, and also any illness that might result from consuming the foods. Mechanical processing likely helped increase energy availability by breaking down cell walls and reducing ingested particle size, which may make food easier to chew and digest. Swallowing a more comminuted bolus, such as when consuming sliced or roasted meat, aids digestion by increasing the surface are over which digestive enzymes can act. Cooking in particular may

further increase energetic gain (e.g.(Boback et al., 2007; Carmody and Wrangham, 2009; Carmody et al., 2011)) by denaturing and degrading cell membranes and proteins (Lewis and Purslow, 1989; Greve et al., 1994a; Ng and Waldron, 1997; Alvarez and Canet, 2001; Lillford, 2001; Tornberg, 2005). Additionally, cooking has the added benefit of reducing endogenous parasites and killing bacteria that are especially prevalent in meat (particularly scavenged meat (Ragir et al., 2000)) and which might cause illness. Reduction of parasitic and bacterial load would also have the added benefit of increasing food storage time, which may have been especially important in highly variable environments where food acquisition outcomes are uncertain.

The data in this dissertation point to another shift that helped increase net energy gain: the reduced cost of mastication (and possibly ingestion). On average, chimpanzees spend nearly 40% of the day chewing foods, while modern humans spend only about 5% of the day masticating (Organ et al., 2011). This extreme difference suggests that food processing must have had major effects on the rate at which calories are ingested, which might have further morphological or behavioral consequences. For example, a recent study by Fonseca-Azevedo and Herculano-Houzel (2012) indicates that the number of feeding hours needed to meet the caloric requirements of hominin large brains (particularly those of later *Homo*) would have been prohibitive without a substantial increase in the number of net calories attained per chew, perhaps facilitated by the adoption of food processing techniques. Although chewing rate depends in part on the mechanical properties of the foods (for example, brittle foods are chewed at a faster rate than more compliant, softer foods (Mioche et al., 1999)), it is possible to use the masticatory data collected in Chapter 3 to estimate the effects of simple Paleolithic food processing techniques on the amount of time that a hominin would spend masticating. When average subject chew number is substituted for masticatory force in the modeling equations outlined in Chapter 3, we see that cooking but not mechanical processing, significantly decreases the number of chews used by *H. sapiens* (Table 5.1); on average approximately 7,000-8,400 fewer chews are used to consume a cooked versus

raw diet, with the greatest decrease occurring when meat comprises a low percentage of the total daily calories (5%). If one assumes that humans chew at a rate of 100 bites per minute (the average rate when chewing raw carrots, raw cucumber, roast pork and soft *Surimi* gel (Kohyama et al., 2007)), one can estimate that humans would spend approximately 70-83 more minutes chewing if they ate only uncooked foods. This represents a significant time savings.

Note, however, that although time and energy spent chewing is reduced, these saving are at least partially countered by increased time spent processing foods. This is especially true of mechanical techniques, which require not only the time and effort to make stone tools, but also necessitate muscular work to process the foods. In contrast, simple cooking such as roasting is a much less involved processing method that typically requires less effort. Except for upfront costs associated with gathering fuel, clearing surrounding brush, and starting a fire, roasting requires little work other than occasionally feeding the fire or turning the food.

Table 5.1. Daily number of chews (in thousands) used by *H. sapiens* consuming raw or processed diets of meat and tubers. [a]

		Number of Chews *(thousands)*		
		Male	*Female*	***Average***
5% Meat 95% Tuber	Raw	39.1	41.6	***40.4***
	Mechanically Processed	40.1	42.8	***41.4***
	Roasted	31.6	32.5	***32.0***
25% Meat 75% Tuber	Raw	36.8	39.8	***38.3***
	Mechanically Processed	37.0	40.0	***38.5***
	Roasted	29.8	31.3	***30.5***
50% Meat 50% Tuber	Raw	33.8	37.4	***35.6***
	Mechanically Processed	33.1	36.6	***34.9***
	Roasted	27.4	29.8	***28.6***

[a] *Estimated number of chews (in thousands) that H. sapiens would use per day if consuming a raw, mechanically processed (average of sliced and tenderized data) or roasted diet of tubers (yams, carrots, and beets) and meat. See Chapter 3 for experimental details. The diets were modeled so that meat comprised a low (5%), medium (25%) and high (50%) proportion of the total daily calories.*

[b] *Number of chews were calculated assuming that the H. sapiens must meet the daily caloric requirements (DCR) estimated by Aiello and Key (2002): H. sapiens male = 2258.5 kcal, female (lactating) = 2664.7 kcal.*
Number of chews = ((DCR X % meat) X (# chews per kcal meat)) + ((DCR X % tuber) X (# chews per kcal tuber))

Future Research.

Research often leads to more questions than answers. I therefore conclude with seven areas of future research that will help to further test and elucidate the many effects that food processing has had on hominin mastication and morphology.

(1) <u>Study additional foods and processing methods</u>. A main limitation of the experiments presented in this dissertation is the small number of foods and processing types that were tested. Future research is needed to consider additional processing methods and foods in order to better understand the diverse effects that food processing has on masticatory performance. For example, early hominins could have used methods such as grinding, maceration and drying, which would have affected mastication, perhaps in varying ways. It is reasonable to predict that grinding foods into small, fine pieces would have significantly reduced masticatory force and average size of swallowed particles, while maceration would have softened the foods and reduced chew force, but because of the extra liquid, a less comminuted bolus would be swallowed ((Hutchings and Lillford, 1988; Prinz and Lucas, 1995, 1997; Lucas, 2004), and see Chapter 3 discussion). In contrast drying foods, especially meat, would likely increase masticatory force production per unit mass. Should drying make meat more stiff and reduce elastic energy loss, however, comminution would be greatly improved and a much more fractured bolus would be swallowed. An additional complication is that food processing techniques are often combined in ways that are hard to detect in the archaeological record. For example, many North American Indian groups consumed pemmican, lean, dried meat that is then ground into a powder and mixed with rendered fat. Further, even when one processing method is used, variations in the material composition of the foods (e.g., cellulose composition of plants, and the amount of fat and collagen in meat) cause foods to respond differently to the processing treatment ((Wheeler et al., 2000; Lucas, 2004; Schonfeldt

and Strydom, 2011; Dixon et al., 2012). More research is needed to explore the effects of the vast array of possible foods and processing treatments.

(2) Material Properties. Material property tests are much less cost and time-intensive to perform than the experiments presented here on masticatory force production and performance. Therefore, one potentially efficient way to assess the masticatory effects of food processing and other dietary changes is to estimate masticatory forces from measurements of the material properties of foods. In order to do this, more experiments are needed to confirm that the strong correlations between tuber material properties and masticatory performance documented in Chapter 3 exist for a broader number of foods and processing types, especially for 'space-limited' foods like meat.

(3) Peak Forces. Another limitation of the masticatory experiments presented in Chapter 3 was the lack of peak force data. While the force impulse is especially relevant to masticatory studies because it is a measure of the total amount of energy used to fracture a food item, masticatory morphology will also be affected by the peak forces that are generated. Unfortunately, peak forces were poorly estimated from the EMG-calibration protocol used in Chapter 3 (r^2 ranged from 0.16 to 0.85) and future studies will have to investigate better methods for calculating peak forces from masticatory experiments. One option is to improve the EMG-force calibrations by having subjects use more dynamic biting motions that mimic natural chew strokes, as opposed to the isometric clenching bites used in Chapter 3. An additional and possibly more accurate technique is to use intra-oral force transducers to measure chewing forces *in vivo* (e.g.,(Hagberg, 1987; Proschel and Raum, 2001; Shimada et al., 2012)). A potentially serious problem with this method, however, is the presence of the transducer and its accompanying wires, which precludes 'normal' chewing and may confound the data. Future methodological designs will need to try and minimize these effects, or at the very least assess the degree to which they bias the results.

(4) Masticatory Kinematics. Future work should assess the effects of food processing on jaw kinematics and kinetics. Only vertical masticatory forces were quantified in Chapter 3 and although these forces provide most of the energy that is used to fracture a food item, horizontal forces are also important in comminution. Jaw movement patterns are altered by the material properties of the foods consumed (Reed and Ross, 2010; Iriarte-Diaz et al., 2011), and it is therefore reasonable to predict that food processing will affect the relative contributions of vertical and horizontal forces to overall food fracture. Additionally, the EMG data collected in Chapter 3 indicate a non-uniform response to chewing processed foods (e.g. Table 3.4 and Table 3.8), which is suggestive of a change in jaw kinematics and therefore force production. By studying the specific effects that food processing has on jaw kinematics, it may be possible to estimate changes to the horizontal force component and better model total masticatory force that is used to fracture food.

(5) Energetics/Time allocation. As mentioned earlier, the use of food processing techniques by hominins would undoubtedly have had a number of energetic consequences, such as increasing the net energy gained from food (e.g. (Wrangham et al., 1999; Carmody and Wrangham, 2009; Wrangham, 2009; Carmody et al., 2011)). In order to fully assess the ways in which food processing affect energy balance, however, research is needed to quantify the number of calories and time spent on different processing activities including costs associated with tool making and fire building, as well as ingestion, mastication and digestion. Recent experiments testing the effects of simple food processing on masticatory and digestive costs in model animals have documented significant energetic savings (approximately 12-50% fewer calories) when consuming ground and/or cooked meat and tubers (Boback et al., 2007; Carmody, 2012).

(6) Functional Integration (Animal Experiments). The preliminary animal experiment described in Chapter 4 needs be replicated, but with a few changes. First, the sample size needs to be increased

and the protocol extended to capture a longer period of development (ideally weaning to adulthood). Extending the duration of the experimental treatment period is especially necessary to quantify the effects of masticatory force variation during development on the resulting adult morphology.

Second, it would be useful to assess masticatory performance in the animals. There are a number of ways in which to assess performance. The simplest would be to quantify time spent feeding, but a more involved, and ultimately more precise way to determine masticatory performance would be to bond a small force transducer to a molar and measure *in vivo* chewing forces as well as the number of chews used to consume a certain volume of food.

Finally, it would be useful to add another treatment group whose masticatory forces are intermediate between the soft, liquefied diet and hard diet. Most animal dietary experiments feed a ground/water-softened diet that profoundly reduces how much masticatory force is necessary (e.g., (Watt and Williams, 1951; Ciochon et al., 1997; Tokimasa et al., 2000; Maki et al., 2002; Larsson et al., 2005). While these diets maximize masticatory force difference between two treatment groups, they may be a poor proxy of the more moderate force reductions caused by simple mechanical processing and cooking methods. Another potential effect of consuming a nearly liquid diet is abnormal tongue movements, which may influence masticatory morphology independent of chewing forces (e.g. (Larsson et al., 2005)). Including a third group of animals that chew foods requiring intermediate levels of masticatory force will address these issues and will help assess how different levels of force influence morphology as a reaction norm. For example, do higher masticatory forces generate progressively greater measures of morphological integration? Or is the relationship between force and integration a threshold effect, in which the system remains integrated as long as a certain amount of minimum force is generated?

(7) <u>Functional Integration (Human Experiments)</u>. There are a number of improvements that should be made to the preliminary human functional integration experiment described in Chapter 4. First, sample size should be greatly increased. This study utilized the masticatory performance data collected in Chapter 3, which limited sample sizes to eight, ten, or fourteen depending on the variables measured. In contrast, most covariation studies of integration require sample sizes of at least thirty to forty to reach significance. A power analysis of the data suggest that a sample size of at least 30 is needed in order to measure a significant relationship between occlusal integration and measures of masticatory performance such as chew number and comminution efficiency ($p \leq 0.05$; 80% power; Simple Interactive Statistical Analysis, www.quantitativeskills.com/sisa).

In addition to increasing sample sizes, future functional integration studies should also maximize masticatory performance differences. In the current study, the relationship between morphology and masticatory performance did not change depending on whether the food was raw or processed. This does not necessarily mean, however, that the "disuse" hypothesis should be rejected. Malocclusions are now epidemic presumably because of the consumption of highly processed foods. It is therefore reasonable to predict that the relatively simple processing methods tested in this dissertation did not affect mastication enough to pick up a signal of low or absent functional integration. Future studies can more rigorously test the "disuse" hypothesis by increasing the differences in predicted performance between the chewed foods. For example, subjects can chew super-processed versus raw foods (i.e. peanut butter and whole, raw peanuts) or chew on artificial test foods with known material properties, such as soft versus hard gels, wax or gum (Iwase et al., 2006; Gambareli et al., 2007; Schimmel et al., 2007; Oueis, 2009; Speksnijder et al., 2009).

A final area for improvement is to use a different measure of morphological integration that might be more relevant to masticatory performance. The PAR score used in the current study has the

advantage of being easy to measure and yielding a single score that continuously grades occlusion from 'perfect' (score = 0) to progressively higher levels of occlusal deviance. The PAR score was developed, however, to assess need the need for orthodontic treatment, and may poorly quantify many occlusal features most relevant to mastication. Additionally, because it was designed to assess occlusion at any dental stage, measurement precision is reduced because only the lowest scoring tooth is counted towards the final aggregate PAR score. To address this problem, I am currently testing other measures of morphological integration that might be more relevant to the masticatory performance variables measured in Chapter 3. One potentially promising avenue of research is to use occlusal contact areas as a proxy for integration. A higher degree of covariation among the teeth and jaws should result in more contact points between the upper and lower teeth, which will improve masticatory performance because the occlusal surface is the proximate site of food fracture (Owens et al., 2002; Buschang, 2006; Iwase et al., 2006).

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