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Nutritional contribution of plant foods to human diet in evolution

Schnorr, S.L.

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Author: Schnorr, Stephanie
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CHAPTER 3

ASSESSING DIGESTIBILITY OF HADZA TUBERS USING A DYNAMIC *IN-VITRO* MODEL

Schnorr SL, Crittenden AN, Venema K, Marlowe FW , Henry AG. 2015. *American Journal of Physical Anthropology*, 158: 371-385. doi: 10.1002/ajpa.22805.

ABSTRACT 2

Bioaccessibility is a useful measure for assessing the biological value of a particular nutrient from food, especially foods such as tubers. The wild tubers exploited by Hadza foragers in Tanzania are of interest because they are nontoxic, consumed raw or briefly roasted, and entail substantial physical barriers to consumers. In this study we attempted to elucidate the biological value of Hadza tubers by measuring the absorption of glucose through *in-vitro* digestion. We quantified digestibility using data from 24 experimental trials on four species of Hadza tuber using a dynamic *in-vitro* model that replicates digestion in the stomach and small intestine. Analysis of glucose in the input meal and output dialysate revealed the accessible glucose fraction. We also conducted assays for protein, vitamin and mineral content on whole tubers and meal fractions. Bioaccessibility of glucose varies depending on tuber species. Holding effects of chewing constant, brief roasting had negligible effects, but high intraspecific variation precludes interpretive power. Overall, Hadza tubers are very resistant to digestion, with between one to two thirds of glucose absorbed on average. Glucose absorption negatively correlated with glucose concentration of the tubers. Roasting may provide other benefits such as ease of peeling and chewing to extract edible parenchymateous tissue. A powerful factor in glucose acquisition is tuber quality, placing emphasis on the skill of the forager. Other nutrient assays yielded unexpectedly high values for protein, iron, and iodine, making tubers potentially valuable resources beyond caloric content.

3.1 Introduction

Bioaccessibility, the portion of food that is available for absorption into the systemic circulation, is a newly appreciated metric in nutritional science and food ecology for assessing the contribution of food towards metabolism (Stahl et al., 2002). Also known as the biological value of food, bioaccessibility (also sometimes referred to as bioavailability) is normally expressed as a ratio or percentage of nutrition absorbed versus consumed and is descriptive beyond nutritional composition because it accounts for digestible and indigestible constituents. Applying this metric to nutritional ecology in anthropology is important because Plio-Pleistocene hominins survived on wild foods, which have not undergone artificial selection for nutritional content and digestibility (Johns, 1996; Purugganan and Fuller, 2009). Wild plants may be especially problematic because natural selection would favor plants that resist consumers, if they do not facilitate reproduction and dispersal, either through physical or chemical barriers (Johns, 1996).

One particular type of wild food, called plant underground storage organs (USOs), is thought to be of importance in hominin dietary ecology. While many types of USOs are a common component of the modern human diet (Couture et al., 1968; Woodburn, 1968; Hart and Hart, 1986; Bradbury and Holloway, 1988; Oke, 1990; Lindeberg et al., 1996; Cordain et al., 2000; Dounias, 2001), their edibility and nutrition is highly variable depending on many factors, such as the species, season, and age of the specimen (Bradbury and Holloway, 1988). As a result, USO processing and consumption is a regionally and culturally specific process. Research in human dietary ecology often questions whether the nutritional needs of human foragers living in East Africa could be met from the consumption of a diet consisting primarily of meat, USOs, and other plant foods (Ungar et al., 2006). USOs were likely a key resource for early hominins because of a number of appealing properties including: 1) wide distribution and high yield throughout a variety of environments (Vincent, 1985; Couture et al., 1986; Oke, 1990; Yasuoka, 2006) possibly enabling survival for early hominins ranging across and out of Africa; 2) a concentration of minerals and carbohydrates that are critical elements for brain growth (Finglas and Faulks, 1984; Bradbury and Holloway, 1988; Aiello and Wheeler, 1995; Ashworth and Antipatis, 2001; Laden and Wrangham, 2005; Wrangham et al., 2009; Deshmukh and Rathod, 2013); 3) recurrent exploitation by women in foraging societies for whom reproductive investment and constraints make hunting an unfavorable subsistence strategy (O'Dea, 1991; Hawkes, 1996; O'Connell et al., 2002; Marlowe, 2005; Berbesque et al., 2011); 4) and finally, USO foraging draws heavily on the experience and allomothering supplied by older post-reproductive female foragers, providing a compelling argument for the importance of grandmothers in human evolution (O'Connell et al., 1999; Crittenden and Marlowe, 2008). A reliance on USOs often implies the need for cooking to breakdown physical and chemical barriers in the plant so that a human consumer can access the nutrition commensurate to what was expended in acquisition (Stahl, 1989; Carmody et al., 2011). The Hadza hunter-gatherers of East

Africa offer a unique opportunity to test the bioaccessibility of nutrition in the wild USOs, specifically tubers from Fabaceae and Convolulaceae, that they consume year round (Marlowe and Berbesque, 2009). In this paper we explore the factors relating to digestibility and glucose absorption using a dynamic *in-vitro* model for four different species of wild tuber regularly consumed by the Hadza.

Image 3. A day's forage is shared back at camp amongst all camp members, including men and older children.

Traditional caloric measurements in nutritional science, based on quantitative assays, portray food in only one dimension. To complete the picture, we must understand the ability and limitations of humans to deal with complex macronutrients, micronutrients, and antinutrients through digestion, especially these latter compounds that occur mainly in plants and adversely affect nutritional absorption. While in theory we may recognize that the digestive tract is not a perfect nutrient harvester, however, in practice it is much harder to quantify the extent to which different food is broken down and absorbed, or passed on into the colon for fermentation (Stahl et al., 2002). Add to that the effect of different food processing techniques on nutrient accessibility, and the whole notion of exacting nutritional gain from food can seem a vexing process of navigating overwhelming complexity. The human body relies on mechanical, chemical, and enzymatic activity to break down many different complex molecules for absorption into the systemic circulation (Hadley and Levine, 2007). Therefore, to mimic these events *in-vitro*, one must account for varying pH, peristaltic movement, time dependent release of digestive enzymes, and most importantly, an absorption barrier or gradient that will inhibit

passage of larger molecules. From this perspective, it is easy to see that both physical and chemical aspects of different foods can affect the accessibility and efficiency of nutrient absorption, and that this may be further modulated by regional nuances both in culture and biology (Johns, 1996). To address such complexity, we use the TNO *in-vitro* gastro-intestinal model (TIM-1), which allows full simulation of human digestion with validated physiological accuracy to *in-vivo* activity (Minekus et al., 1995, Souliman et al., 2006). The TIM-1 has been validated for nutrients in several types of foods that are generally consumed, and validation has been performed for carbohydrate, protein, and fat digestion in a number of different whole foods (Minekus 1998; see Chapter 7). In addition, vitamin bioaccessibility has been validated for both water and fat-soluble vitamins. In all cases, the TIM-1 results closely correspond to clinical data (Minekus 1998). Therefore, it can be concluded that the system mimics the dynamic physiological process of digestion, and can be used with confidence for unknown meals, including wild foods.

Food processing is an incredibly important aspect of human dietary ecology (Stahl, 1989; Wollstonecroft et al., 2008). Historically, humans have overcome refractory or toxic compounds in food through simple or complex processing techniques that can cause physical or chemical changes to food properties. The archaeological precedence for these techniques is still not clearly understood, but certainly physical processing, such as cutting or pounding, has been in use since at least 2.6 million years ago, with good evidence for nearly one million years prior to that (Semaw et al., 2003; McPherron et al., 2010; Lemorini et al., 2014). Cooking, in particular, is a profoundly important technology for modern humans and it is proposed that the adoption of cooking by human ancestors has led to a universal human dependency on cooked foods (Wrangham and Conklin-Brittain, 2003; Carmody et al., 2011). However, evidence of fire in an archaeological context, whether implying a consistent presence or use in cooking, is still debated (Goren-Inbar et al., 2004; Roebroeks and Villa, 2011; Berna et al., 2012). Cooking breaks down complex molecular structures such as long-chain polysaccharides and proteins, making food softer, and exposing binding sites for digestive enzymes, which facilitates absorption in the gut (Englyst and Cummings, 1986; Holm et al., 1988; Bornet et al., 1989). In addition, thermal treatment of food, especially plants, can denature toxic compounds or enzymes responsible for releasing toxins, as well as kill pathogens, making food safe for human consumers (Stahl, 1984; Johns, 1996). For a human forager, cooking reduces pathogen load and can help buffer against food shortages from seasonal or climatic effects, niche competition, and marginal habitat occupation by enabling expansion of dietary breadth. Therefore, if the control of fire incurred limited costs in terms of time, energy, and resource management, then its use for cooking was clearly advantageous to hominin survival.

While many USOs are toxic when raw, most notably common cultivated yams such as *Dioscorea alata* and *D. rotundata*, cassava (*Manihot* spp.), and potato (*Solanum tuberosum*) (Bradbury and Holloway, 1988), the wild tubers exploited by the Hadza are all non-toxic and are consumed raw as well as cooked (Woodburn, 1968; Vincent, 1985). Instead of toxins, Hadza tubers maintain defense

against consumers through largely physical attributes. Edible parts are found deep underground, covered in a thick inedible bark-like skin, the peridermis and cortex, and are laced with inedible fibers to which the edible parenchymatous tissue (the pulp) adheres, necessitating considerable time and dedication to extract and consume (Schoeninger et al., 2001). The Hadza forage and consume these wild tubers year-round, which constitutes a significant portion of their diet, supplementing other foods such as game meat, fruits, legumes, and honeycomb (Marlowe and Berbesque, 2009). Roasting times reportedly range between three (Dominy et al., 2008) to thirty minutes, with the lower end of this range, described as "light roasting" (Tomita, 1966) and resulting in "lightly charred" tubers (Woodburn, 1966), cited as approximately five minutes (Woodburn, 1968; O'Connell et al., 1999; Mallol et al., 2007; Marlowe and Berbesque, 2009). Many researchers have questioned why the Hadza bother roasting the tubers for such brief durations (Marlowe and Berbesque, 2009), and how these roasting practices may affect nutritional gain from these tubers. The brief roasting technique employed by the Hadza to consume their tubers is a simple form of cooking, requiring only an open-flame fire, and no other extended processing common to plant food preparation such as grinding, leaching, or sprouting (Stahl, 1989). As such, on the basis of putative stone tool technologies and cranial architecture of early hominins, brief roasting may fall within the behavioral and cognitive capacity of early *Homo* (Neubauer and Hublin, 2012). Modern hunter-gatherers should not be mistaken as Paleolithic emulations, but rather referents to the behavioral and nutritional adaptations that may have been necessary for early hominin occupation of the East African savanna-mosaic environment. Therefore, it is of interest and relevance to anthropologists modeling hominin nutritional ecology to understand the extent to which the constituents of edible East African tubers become available through digestion to the consumer's metabolic physiology.

This study entails the first attempt to quantify the digestibility of wild East African tubers exploited by a modern population of hunter-gatherers. We analyzed the glucose absorption and protein and vitamin contents of four species of tubers consumed by the Hadza, in both raw and briefly roasted states, using the TIM-1 dynamic *in-vitro* model of the human gut (Minekus et al., 1995). Glucose absorption represents the bioaccessible fraction, and glucose bioaccessibility was assessed across and within tuber species for differences between cooked and raw specimens. Our findings indicate large intraspecific variation, which reduces statistical power, but in general that cooking has variable effects, depending on the species, and that tubers were highly resistant to digestion, resulting in low glucose accessibility.

3.2 Methods

3.2.1 Field collections

Tuber collections were conducted during the rainy season in January 2013 in two different camps in Yaeda Valley, near Lake Eyasi, northern Tanzania. Camps were selected on the basis of having at least 10 adults, both men and women, with women foraging daily for tubers. Upon arrival into the camp, members were verbally informed of the study and asked whether or not they would like to participate before researcher presence was established. All data were collected with research permission from the Tanzanian Commission for Science and Technology (COSTECH), permit number 2012-315-NA-2000-80. We collected tubers during foraging trips in the mornings and evenings over a period of four to five days at a time. We only obtained tuber species that the women were actively gathering for consumption. In the end, tubers present in sufficient quantity to complete the study included five main species that are regularly consumed by the Hadza, which is summarized in Table 1: *//ekwa* (*Vigna frutescens*), *shumuko* (*Vatovaea pseudolablab*), *mak'alitako* (*Eminia entennulifa*), *panjuko* (*Ipomoea transvaalensis*), and *penzepenze* (*Vigna* sp.). The women were asked to forage as normal and only tubers offered willingly to the research team were used for subsequent analysis. In order to avoid taking all the foraged food (which was offered), and to check whether offered tubers were actually worth consuming and not just sub-optimal tubers, we would randomly reject an offered tuber and observe whether the women kept or discarded it. In all cases, the tubers were kept and consumed.

Image 4. Collecting tubers with Hadza women meant carrying my share of the load of willingly offered forage.

Women were observed during roasting sessions in camp and measurements of tuber circumference and roasting time recorded for available species brought back to camp for reference (Table 2). Once back in camp, collected tubers were immediately cleaned of adherent dirt and soil with a dry towel and toothbrush, weighed using a hanging balance (KERN HBD-N), and then stored raw, whole, and intact in a dark, dry, breathable container at ambient temperature in the shade for no more than six days while in camp.

Image 5. A large haul of *I. transvaalensis* tubers and one long *V. frutescens* root from an afternoon forage.

This period of time in a warm, humid, but ventilated container is an important step in tuber preservation, constituting a curing phase that lasts from five to 20 days. This is the standard procedure recommended by the Food and Agriculture Organization of the United Nations (FAO) for tropical tuber crops that are shipped world-wide for human consumption (Diop, 1998; see Chapter 3, section 3.1.1), and is the ideal storage procedure for our purposes of simulating human consumption and digestion. The curing phase allows roots and tubers to heal surface wounds and strengthen the peridermal tissue when held at relatively high temperatures and humidity for some days after harvest. This also helps mitigate water loss, cellular respiration, and invasion by pathogens (Diop, 1998). During the time of our visit, temperatures recorded at the nearest weather station in Arusha, TZ ranged between 13°C and 31°C for the daily lows and highs, with an average high temperature of 28°C, and average high humidity between 70-90%. Optimal conditions for curing include temperatures between 15-40°C and relative humidity at 85-90% (Diop, 1998; see Chapter 3, Table 3.1). After a maximum of six days in our field camp, tubers were transported in the container to a cool ventilated storage shed for the remaining duration of our field work (a maximum time of one week) until we could transport all tubers directly on the airplane back to our labs in Leipzig, Germany. Once we arrived at the lab, all tubers were immediately refrigerated at 4°C, the recommended temperature for long-term storage, until further processing (a maximum of one week). Throughout this time, the condition of the tubers was closely monitored, and those that showed major damage such as cuts or bruises were not accepted. Cutting and attempting to air-dry samples in the field introduces numerous mechanistic vectors for loss of nutrition such as from

sun scorch, microbial contamination, and insect infestation (Diop 1998; see Chapter 2, Table 2.1). No characteristic signs of rot were detected by sight (mold, browning, bruising), smell (acrid or very sweet), or taste (sour or acidic) in all 68 tubers except for two specimens, which were realized to have sustained surface damage and subsequently discarded (see Figure 1).

Table 1. Taxonomic identifications of Hadza tuber species with brief physical quality descriptions.

Latin name	Hadza name	Physical traits
Vigna frutescens	*//ekwa*	Elongated tuber with woody bark-like peridermal and cortical tissue. Parenchyma adheres to thick longitudinal inedible fibers that are chewed and expelled.
Eminia entennulifa	*mak'alitako*	Large round tubers with thinner cortical peel than *V. frutescens* but consisting of cortical layers and a juicy parenchyma filled with small thin inedible fibers.
Ipomoea transvaalensis	*panjuko*	Smaller tubers similar in shape and size to *I. batatas* (sweet potato). Peel is thin and no inedible fibers restrict consumption. Most commonly consumed raw.
Vatovaea pseudolablab	*shumuko*	Large round juicy tuber with thick layered cortical tissue and very fine internal fibers.
Vigna sp.	*penzepenze*	Elongate and fibrous, similar to *V. frutescens* but slightly smaller and with distinct nodules. Cortical layer is thinner but difficult to remove without a knife or fire.

Table 2. Field measurements of circumference and cooking times for *V. frutescens* and *V. pseudolablab*.

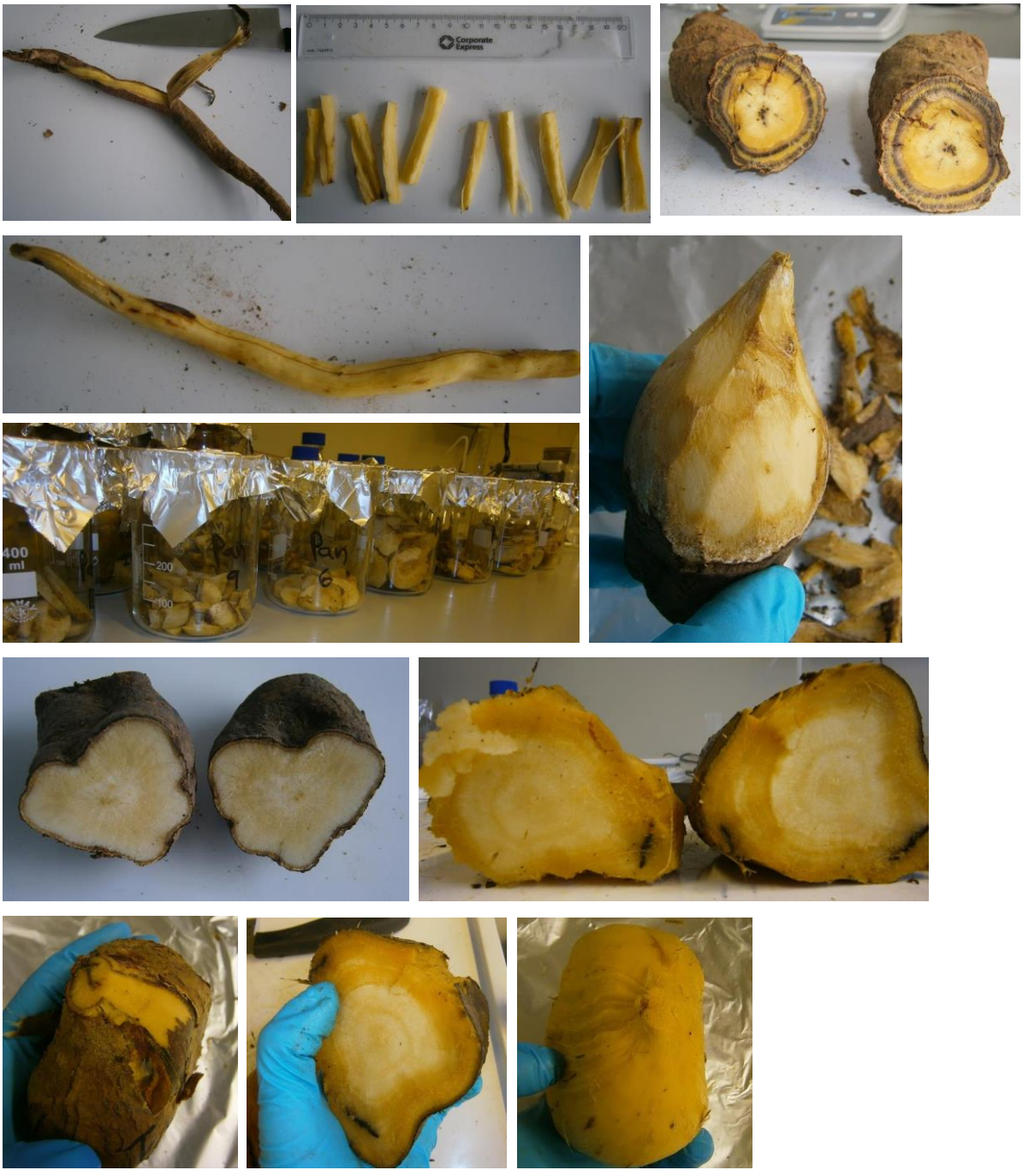
Specimen	Circumference (cm)[1]	Cook time (min)[2]	Notes[3]
V. frutescens 1	13.34	7	moved at 3 mins, only distal end in fire, first half out
V. frutescens 2	11.43	5	moved at 3 mins, only distal end in fire, first half out
V. frutescens 3	18.42	5	
V. frutescens 4	15.88	5	turned after 2 minutes and after 3 minutes
V. frutescens 5	13.97	5	
V. frutescens 6	10.80	13	turned at 4 and 5 mins, only ends in fire at 9 mins
V. frutescens 7	12.07	13	turned at 4 and 5 mins, only ends in fire at 9 mins
V. frutescens 8	7.62	9	turned at 4 and 5 minutes
V. frutescens 9	10.80	13	turned at 4 and 5 mins, only ends in fire at 9 mins
V. frutescens 10	10.16	11	turned at 4 and 5 mins, only ends in fire at 9 mins
V. frutescens 11	10.16	11	turned at 4 and 5 mins, only ends in fire at 9 mins
V. frutescens 12	12.07	13	turned at 4 and 5 mins, only ends in fire at 9 mins
V. pseudolablab 1	31.12	6	turned at minutes 3 and 4
V. pseudolablab 2	41.28	10	turned at minute 5
V. pseudolablab 3	26.04	5	

[1] Circumference was measured at the widest section of the tuber.

[2] Average cook time is 8.73 minutes, not accounting for adjustments that removed a portion of the tuber outside of the fire, and is therefore a conservative calculation.

[3] We observed that tubers are not static during roasting and are often turned or removed from the fire to reposition or reorient the tubers so that all portions are evenly roasted. This means that cooking times can be errantly overestimated if only one half of the tuber is roasted at a time, as is often the case for the longer *V. frutescens* tubers.

a.



b.

c.

Fig 1. Uncooked (**a**) and cooked (**b**) tuber specimens brought back from Hadza land show no characteristic sign of rot, except for two tubers (**c**), which were subsequently discarded.

3.2.2 Roasting and pre-processing

We conducted the roasting and pre-processing (chewing) phase in the plant foods research lab at the MPI-EVA in Leipzig, Germany. The tubers consumed by Hadza are largely composed of tough outer peridermal "peel" and internal inedible fiber, necessitating considerable pre-processing to extract the edible parenchyma. After peeling and chewing for approximately thirty seconds to three minutes, Hadza expel a mass of the inedible fibers, termed a "quid" (Schoeninger et al., 2001). The peel and quid are not subject to gastro-intestinal (GI) digestion, posing a unique challenge for the TIM-1 system, and were therefore removed through peeling and processing in a homogenizer, or "stomacher" (Seward stomacher 400C, Fisher Scientific), prior to our *in-vitro* digestion simulation. Normally, an immersion blender is used to completely liquefy TIM-1 input meals followed by incubation with α-amylase. Therefore, we chose the methods of Schoeninger et al. (2001) as it is the only study of Hadza tubers that fully appreciated and addressed this biologically relevant aspect of Hadza tuber consumption. We sought to replicate these methods for the chewing phase because they allowed us to control the α-amylase type and strength, and manually separate the edible parenchyma from indigestible fiber. The normal TIM-1 protocol was reinstated just before each trial run in which the samples were liquefied by immersion blending to allow passage through the TIM-1 chambers.

Tubers were weighed in the lab using the same hanging balance to determine moisture loss during storage and transit. The only two rotten tubers were discarded. When not in immediate use, specimens were stored at 4°C. Specimens were sorted by species into raw and roast groups, matched by weight, and the roast group was cooked on a high-flame open fire for five minutes (Table 3). This fire was produced to simulate a Hadza tuber roasting fire (bush fire) using small dry wood-sticks with a high initial flame and lasting about 10-15 minutes (Mallol et al., 2007). The temperature of the fire (500-700 °C) and duration of roasting time (5 min) are based on personal (see Table 2) and previous field observations (Woodburn, 1968; O'Connell et al., 1999; Mallol et al., 2007; Marlowe and Berbesque, 2009; for temperature data see Dominy et al., 2008; Table 3).

Table 3. Fire temperatures for simulated roasting trials to emulate Hadza tuber roasting fires.

Trial	Time (min)	Fire Temp °C (i)	Fire Temp °C (o)
1	5	550	600
2	5	550	600
3	5	500	600
4	5	500	590
5	5	500	590
6	5	600	700

Image 6. Setting up and carrying out tuber roasting emulations in Germany during a February snow.

Tubers were peeled by hand to ensure removal of the inedible peridermal and cortical tissues and the resultant peel weighed. The remaining parenchymal tissue was chopped coarsely and combined with water equal to the predetermined moisture loss. The chopped tuber and added water were transferred to a stomacher bag and combined with 50ml warm water (40°C) and 1000U α-amylase (Sigma A1031) per 100g tuber sample. This level of amylase activity was selected to approximate the estimated protein expression level for Hadza derived from a regression of the work of Perry et al. (2007) in their Figure 1C and Supplementary Table 1. Chewing was approximated using the stomacher at 250rpm for two 3-minute intervals. Homogenized tubers were placed on ice immediately for a minimum of 30 minutes to deactivate enzymes, and then fibers constituting the quid were removed manually, weighed, and frozen.

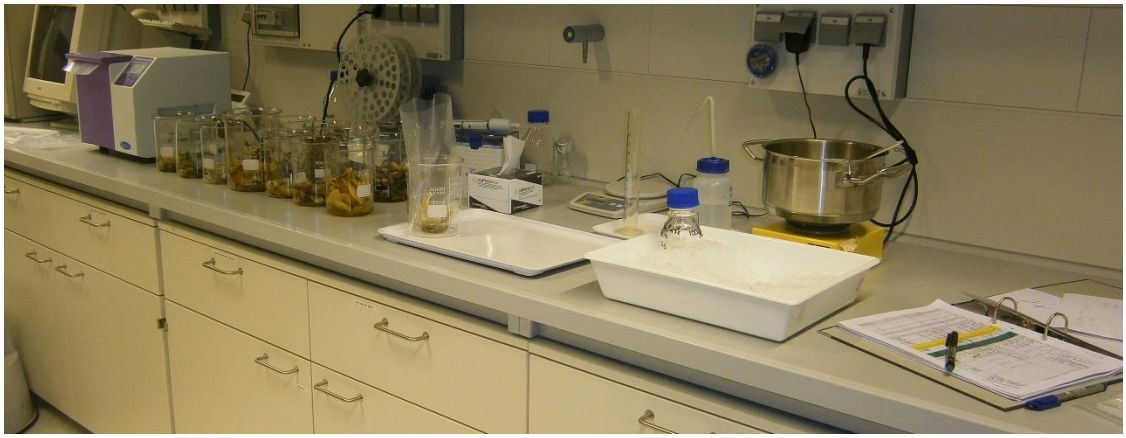


Image 7. Lab set up at MPI-EVA for the pre-processing steps to simulate chewing.

Final tuber samples deemed as the consumable portion were again weighed and then frozen at -20°C until further use. Because *I. transvaalensis* does not contain the same fiber mass and did not properly homogenize in the stomacher, these specimens were simply peeled and blended with an immersion blender and then incubated with α-amylase just prior to *in-vitro* digestion. All peeled and pre-processed specimens were brought on dry ice directly to TNO Department of Pharmacokinetics & Human Studies in Zeist, The Netherlands, to conduct the *in-vitro* digestion trials. All work was conducted by wet weight because this is necessary in the context of our work on the *in-vitro* system and in modeling human consumption of whole foods.

3.2.3 Dynamic *in-vitro* digestion

The TIM-1 system (**T**NO *In-vitro* gastro-intestinal **M**odel) is a unique, validated, computer-controlled simulation of the stomach and small intestine with great reproducibility and application in studies of human health (Minekus et al., 1995, Minekus, 1998; Souliman et al., 2006). A detailed description of the TIM-1 system and methodology has been previously reported (Blanquet et al., 2004; Minekus et al., 2005; Faessler et al., 2006). The model consists of four chambers representing the stomach, duodenum, jejunum, and ileum respectively (Figure 2). Meals comprising the amylase-treated tubers were inserted directly into the stomach compartment of the model and subsequently digested for six hours at 37°C. Peristaltic valve pumps, mixing, transit time, and fluid secretions were regulated automatically by preset values to mimic biological standards. As the introduced meal reached the jejunum and ileum compartments, contents were pumped across two semi-permeable membranes (hollow fiber limited to ca. 5kDa) that removed water, simple sugars and amino acids (products of digestion), through which the dialysate fluid was pumped at a rate of 10ml min^{-1}. This removal prevented build-up of metabolites that otherwise would inhibit further enzyme activity. Membrane absorption, dialysate and ileal delivery were collected every hour for sampling throughout the six hour run.

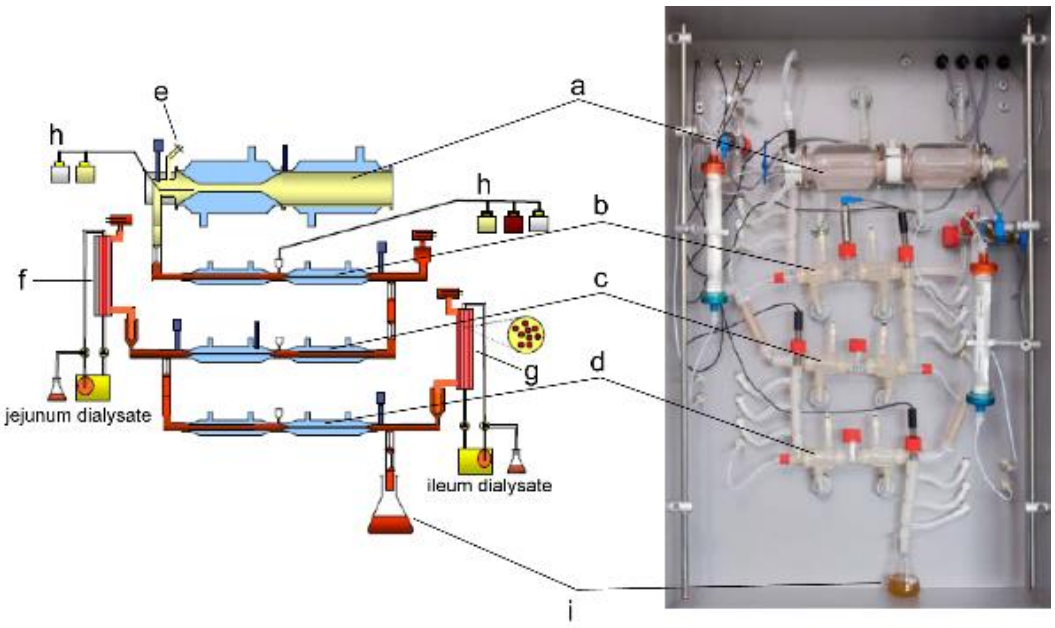


Fig 2. Schematic of the TIM-1 system illustrating the four main compartments: stomach (a), duodenum (b), jejunum (c), and ileum (d). Meal input begins at the stomach port (e), bioavailable nutrients are "absorbed" by passing the jejunum (f) and ileum (g) filters and collected in dialysate reservoirs to prevent buildup of metabolites. Acid and bicarbonate are used to control the pH, following preset values in each compartment while digestive fluids are secreted continually in the small-intestine during the experiment (h). Finally, the ileal efflux (i) is collected and analyzed, representing the ileo-caecal valve where residual meal contents unavailable for absorption would enter the colon. Image adapted from TNO TIM-1 reference manual, (Lelieveld et al., 2009).

3.2.4 Gastric and intestinal environment

The TIM-1 system was prepared prior to each experiment by rinsing and loading the compartments with starting fluids, following standard procedures (Faessler et al., 2006). The duodenal compartment was filled with 55ml of 50% bile solution (4g porcine bile in 250g water; Sigma B8631), 25% pancreatine solution (supernatant of 17.5g in 250g water, centrifuged 20 min at 9000 RPM at 4°C; Pancrex V, Paines and Byrne, Greenford, UK), 25% small intestine electrolyte solution (SIES; composed of NaCl 5g l^{-1}, KCl 0.6g l^{-1}, $CaCl_2 \cdot 2H_2O$ 0.3g l^{-1}, $NaHCO_3$) and 1g trypsin (Sigma T9201). Jejunal and ileal compartments were filled with 100ml of SIES. The pH was controlled in the stomach along pre-set values of 5.2, 3.2, 2.2, and 1.7 at 0, 30, 60, and 120-360 min, respectively, by secretions of 1M HCl or water. In the small intestine, the pH was held at 6.2, 6.5 and 7.4 in the duodenum, jejunum and ileum compartments, respectively, by secretions of 1M $NaHCO_3$. The model was allowed to heat to 37°C in all compartments prior to the experimental runs.

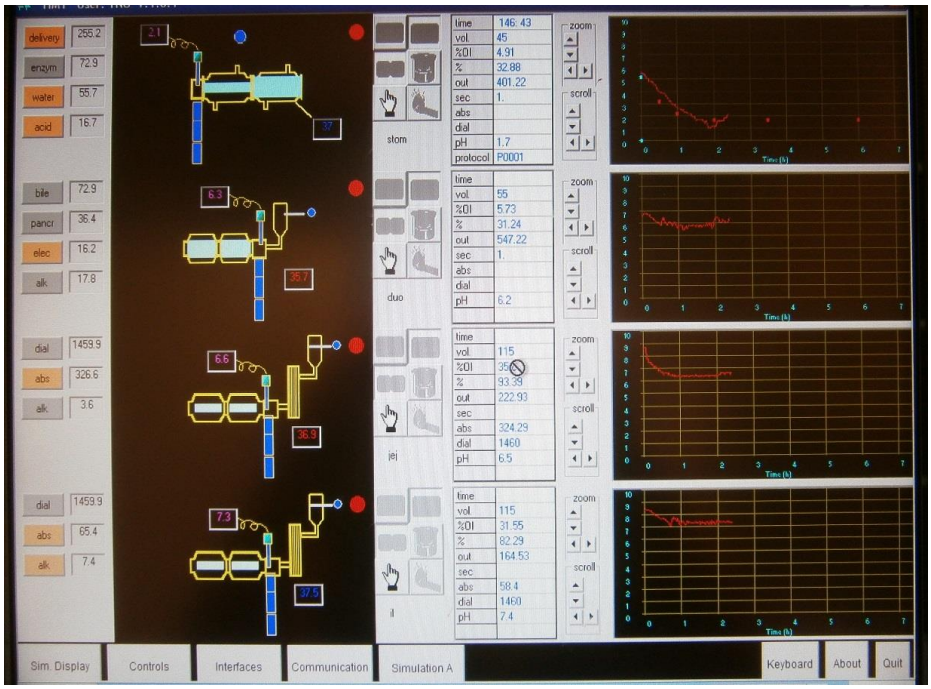


Image 8. The computer controlled TIM-1 interface showing preset values in action after start of a run.

3.2.5 Meal preparation

Meal preparation was handled according to standard procedure (Blanquet et al., 2004; Dominy et al., 2004). Initially, individual amylase-treated tuber specimens were run in duplicate for both cooked

and raw test states except for *I. transvaalensis*, which had to be combined due to small individual sizes. However, for some technical replicates we used multiple specimens because the material from one specimen was not enough to meet the minimum input amount for the TIM-1. A third trial was conducted using the remaining consolidated material, portioned by species and cooking state, to attempt to minimize effects from intra-species variation. All tuber samples and the specimen run schemes are detailed in Supplementary Table 1 (see Appendix A). Meals consisted of 200g of amylase-treated tuber, 30g gastric electrolyte solution (NaCl 3.0g, KCl 1.1g, $CaCl_2$ 0.14g dissolved in 46.8g water and adjusted again to 500ml with water), 20g water, 5g SIES, 1M HCl to adjust the pH to 5.2 and 45g rinse water for gastric introduction. For the nutritional reference values, one 2ml and two 1ml meal samples were saved and frozen immediately at -20°C prior to the experimental run.

Image 9. Loading the stomach compartment with the tuber meal and removing all excess air in the membrane.

3.2.6 Sampling and analysis

Digestion of tubers was simulated across six-hour experimental runs, mimicking a period in

which approximately 95% of the meal had transited the complete upper GI tract. At each hour, dialysis bottles were replaced and the absorption, dialysate and efflux weighed and 1ml samples saved to obtain digestion kinematics over time. Samples were stored at -20°C. At the end of each experiment, the residues remaining in the model were removed, weighed and 1ml samples stored at -20°C. The residues and ileal efflux are elements of the meal considered to be inaccessible for digestion and absorption in the small intestine while the dialysate represents the bioaccessible nutritional constituents (Faessler et al., 2006).

Dialysate samples were tested for glucose with glucoamylase (AMG) treatment using a commercial kit (Roche) on a Cobas Mira plus autoanalyzer (Roche, Almere, The Netherlands). With AMG treatment all small and easily degradable dextrins (maltose, maltotriose, and starch fragments with 1-4 and 1-6 bonds) are degraded by AMG. However, more complex molecules such as native starch can be missed by AMG. Therefore, the meal (the input volume consisting of pre-processed tuber plus the meal starting residues and amylase solution) and ileal efflux samples were first submitted to acid hydrolysis through treatment with 1M H_2SO_4 in order to degrade more resistant glucose polymer units that may be missed by standard AMG treatment. Since our aim was to model stomach and small intestine digestion, and because humans can only produce amylases specific for alpha linked glucose polymers, we did not assay for fructose as a product of inulin digestion, which is instead fermented by colonic microbiota (Roberfroid, 1999).

In addition to glucose, we were also interested in the protein content and digestibility of the tubers. We therefore measured amino acids by alpha-amino nitrogen (AAN) analysis using the modified Biuret assay (Noll et al., 1974) on dialysate and ileal efflux samples. A modified Biuret assay better accounts for native proteins using bovine serum albumin (BSA) as a standard and implemented on a Cobas Mira plus autoanalyzer. The total Kjeldahl nitrogen (TN) content of the meal samples was also measured so as to account for all sources of nitrogen (nitrate, nitrite, ammonia and organically bonded nitrogen). A standard calibration curve is generated by boiling known quantities of BSA for five minutes in 1M NaOH. After cooling, Cu^{2+} ions are added (copper sulphate solution), mixed and centrifuged. The absorbance of the supernatant is measured at A500nm and the crude protein concentration curve generated. The same procedure is applied to the test samples and the total protein concentration estimated with the calibration curve. Dividing the protein concentrations by 6.25 results in the calculated TN amount for the unknown samples and a correlation curve is generated with TN (Kjeldahl) and TN (Biuret) measured samples, allowing calculation of the actual TN of the test samples. All analyses were performed by Bio-aNAlytiX (Mook, The Netherlands).

3.2.7 Micronutrient analysis

To date there has been no attempt to quantify the micronutrient content of Hadza tubers. Since this is an important determinant of food quality, we made preliminary assessments of the mineral content of select "meal" doses from the consolidated (within species) samples and the mineral and vitamin content of whole tubers from one species, *penzepenze* (*Vigna* sp.) that were not submitted to *in-vitro* digestion. Ideally we would also analyze the dialysate to obtain measures of micronutrient digestibility, but practicality deemed that establishing baseline values for micronutrients was the first priority. During meal preparation, ~15ml aliquots of each meal (with starting residues) were withdrawn and frozen immediately at -20°C in opaque containers for further analysis. One aliquot was saved for each raw and cooked specimen of the consolidated material, totaling eight meal analyses in all. Because the meals were made up of specimens that had been pre-processed in the form of peeling, pulverizing, exposure to enzymes and one freeze-thaw cycle, we expected that some water-soluble vitamin concentrations could be altered, and so only minerals are reported with confidence for meals (Reddy et al., 1999). The *penzepenze* tubers were left whole and frozen immediately at -20° upon arrival from Tanzania to our laboratory in Germany. They were not used for digestion trials because of insufficient material. However, these specimens were ideal candidates for full micronutrient assays, including vitamins and minerals, because of very limited handling, and can provide some initial estimate of the micronutrient content of some Hadza tubers, namely those also belonging to *Vigna* (e.g. *V. esculenta*, *V. frutescens*, and *V. macrorhyncha*) (Marlowe, 2010), in the legume family, Fabaceae. All analyses were performed by TNO Triskelion, Zeist, Netherlands. Specific analytical methods are reported in full in Supporting Information.

3.2.8 Statistical analysis

Data were compiled and analyzed using Microsoft Excel and R (version 3.0.1) (RCoreTeam, 2013). Kendall rank correlation coefficients were determined using the [cor] function in the R 'Stats' package and visualized using the [pairs] function. Correlation heat maps were drawn using [levelplot] function in the 'Lattice' package (Sarkar, 2008). Significance testing was accomplished using the [cor.test] function with the confidence level threshold set at "0.95". All mean values are reported with ± standard deviation.

3.3 Results

Data obtained from the experimental *in-vitro* trials to measure absorption of glucose from Hadza tubers provide interesting and unexpected results. Where the term "glucose" is used, we recognize that this encompasses free and starch-derived glucose. However, our quantification does not differentiate between free glucose and starch-derived glucose (measured as glucose after acid hydrolysis and AMG) and so we refer to both simply as "glucose" to be in keeping with our direct measurements. Glucose bioaccessibility was assessed by the amount of glucose absorbed relative to the total amount measured in the meal volume and is therefore a percent value. When we compare percent bioaccessibility of glucose with the absolute gram amount of glucose absorbed, we obtain an indication of not only digestibility but also of potential caloric contribution from tuber glucose to the diet. Furthermore, one unique aspect of the TIM-1 dynamic *in-vitro* system is the realistic peristaltic movement and transportation of food material through each anatomical chamber of the GI model. With this feature we are able to witness the digestion process over time, termed the kinetic properties of digestion, which informs us about the rate of passage and how readily nutrients are liberated by digestive enzymes.

3.3.1 Tuber physical properties

In total, four species of tuber were acquired in sufficient quantity for analysis using *in-vitro* digestion: *V. frutescens*, n=11 tubers; *E. entennulifa*, n=10; *V. pseudolablab*, n=14; *I. transvaalensis*, n=31 (Supplementary Table 1). During pre-processing to simulate peeling and chewing, fresh weight, peel weight, fiber weight and resulting edible fraction were recorded to understand how these features may affect the nutritional quality of wild tubers (Fig. 3 and Supplementary Table 1). *Vigna frutescens* has the lowest average edible fraction (26% ± 8%) with the majority of its total fresh weight deriving from the inedible peel and quid fiber. The edible fractions of *E. entennulifa* and *V. pseudolablab* comprise on average half of the total tuber fresh weights (49% ± 11% and 52% ± 10% respectively) with peel and then fiber making up the remaining fraction. The edible fraction of *I. transvaalensis* is 73% ± 7% and dependent only on peel weight since it contains no inedible fiber. As a result, consumption of *V. frutescens* is to a greater extent inhibited by the presence of tough physical barriers to the edible fraction than the other commonly consumed wild tubers in the Hadza diet.

Image 10. The peel from one *V. frutescens* specimen.

3.3.2 Kinetic data

Absorption curves over the six hour duration of the TIM-1 experiments are shown in Figure 4 for each tuber species. Peak glucose absorption occurs during the second hour for all runs except raw *I. transvaalensis*, which instead peaks at the third hour, indicating a much more protracted rate of digestion (first column). The cumulative percent of glucose absorption (second column) is not predictive of the cumulative absolute amount of glucose absorption (third column), due to large differences in initial glucose content of the tubers (discussed further below). In addition, we observe wide variation in glucose absorption regardless of cooking treatment. No clear or consistent pattern is seen with regard to cooking. Finally, except for *V. frutescens*, glucose absorption values are not consistent within or across species, indicating large variability in glucose accessibility and absolute absorption between individual specimens.

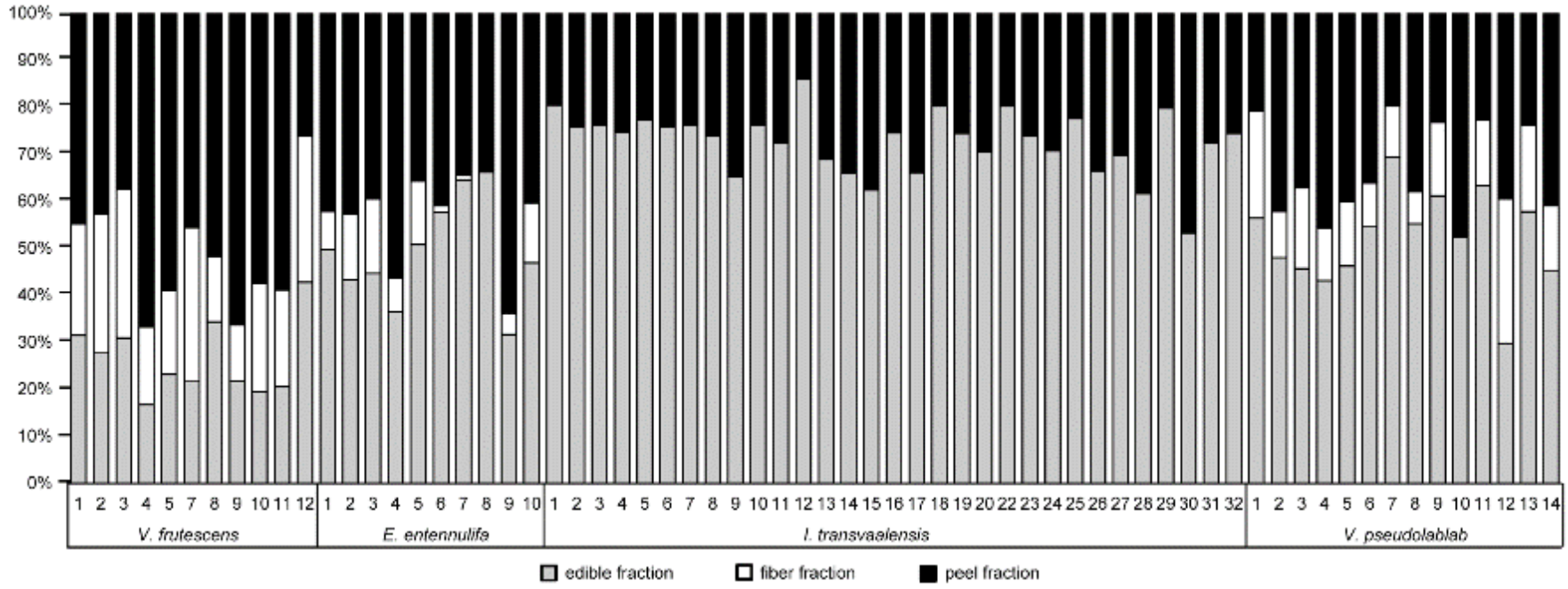


Fig 3. Summary of physical properties of Hadza tubers. Tubers were peeled and processed to remove inedible fiber, called the quid, and extract the edible portion. Each component was weighed to obtain the relative proportion of peel, fiber and edible pulp for different species of tuber. Note that *I. transvaalensis* does not contain inedible fiber.

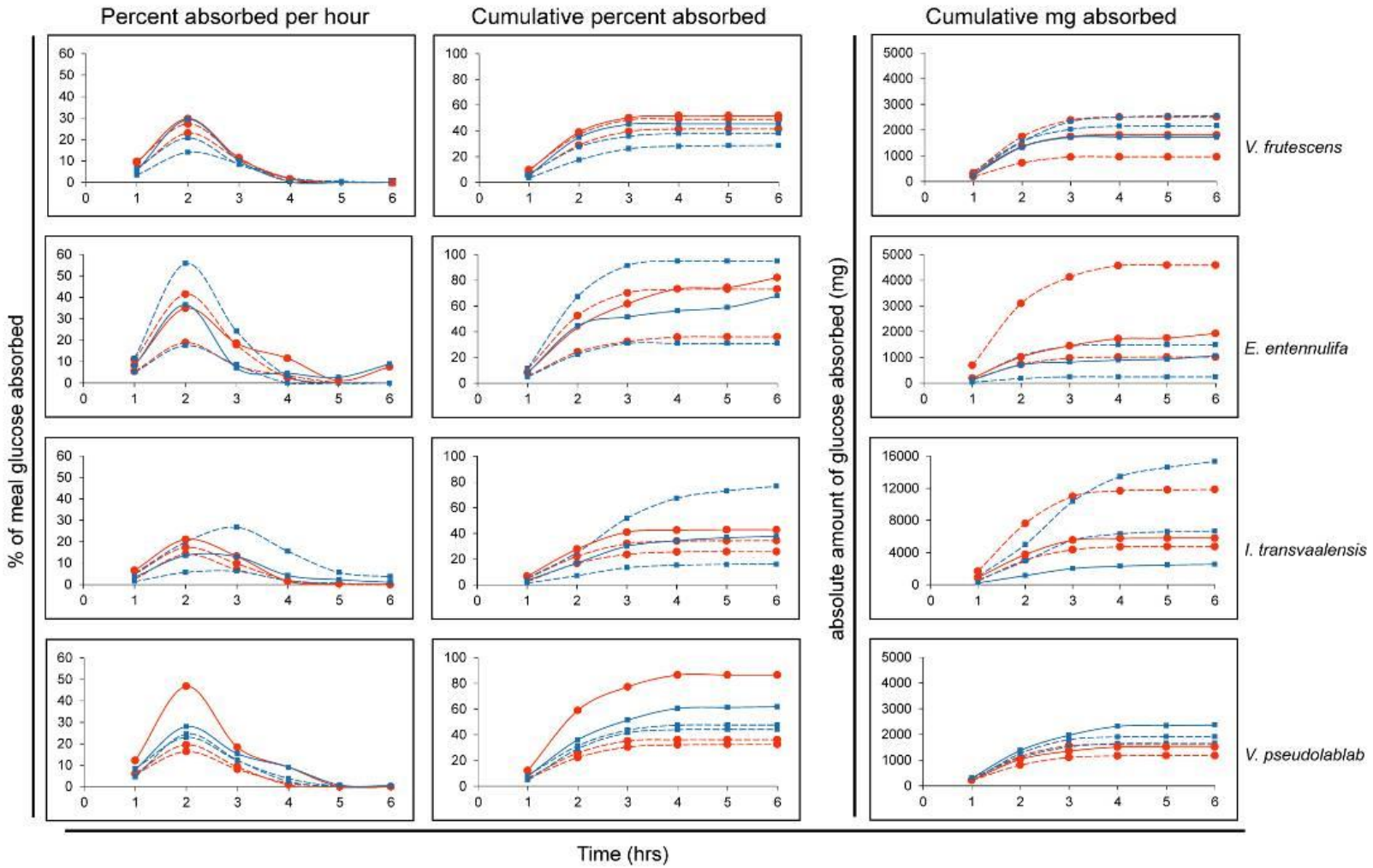


Fig 4. Kinetic data for TIM-1 runs, shown here by tuber species (right axis) with six runs per species; three raw runs (blue squares) and three cooked runs (red circles). Solid lines indicate runs from pooled samples. Columns depict the percent of glucose absorbed at each hour, the cumulative percent of glucose absorbed at each hour, and the cumulative absolute amount (measured in milligrams) of glucose absorbed at each hour, respectively.

This observation is consistent with previous work that demonstrates great variability in the nutritional concentration of Hadza tubers, which is normally attributed to their being non-cultivated wild plants (Vincent, 1985; Schoeninger et al., 2001). Our data now demonstrate that, like initial concentration, digestibility of glucose derived carbohydrate in Hadza tubers is also extremely variable.

3.3.3 Nutritional properties

A summary of the starting glucose and crude protein (TN measured in meals and multiplied by 6.25) concentrations as grams per kilogram of edible wet weight for each run specimen is provided in Figure 5. These values represent the input meal concentration of glucose and protein for each individual *in-vitro* digestion run. On average, *I. transvaalensis* has the highest glucose concentration (78.45g kg^{-1} ± 40.34), followed by *V. frutescens* (26.08g kg^{-1} ± 13.03), *V. pseudolablab* (13.97g kg^{-1} ± 3.96) and finally *E. entennulifa* (12.62g kg^{-1} ± 15.21) (hatched bars in Fig. 5a). Protein values were highest on average for *V. frutescens* (25.63g kg^{-1} ± 11.02) followed by *V. pseudolablab* (21.02g kg^{-1} ± 13.19), *I. transvaalensis* (20.25g kg^{-1} ± 8.55) and finally *E. entennulifa* (11.97g kg^{-1} ± 9.55) (hatched bars in Fig. 5b). We note that glucose values may not be representative of the total carbohydrate content of the tubers since other simple sugars, such as fructose or galactose, were not directly measured. In addition, these averages summarize a very large range of variation within species, as indicated by their standard deviation values, and so it is difficult to resolve an average value of calories from glucose-based carbohydrate for Hadza tubers without sampling many more specimens across both time (season) and space (geographical location).

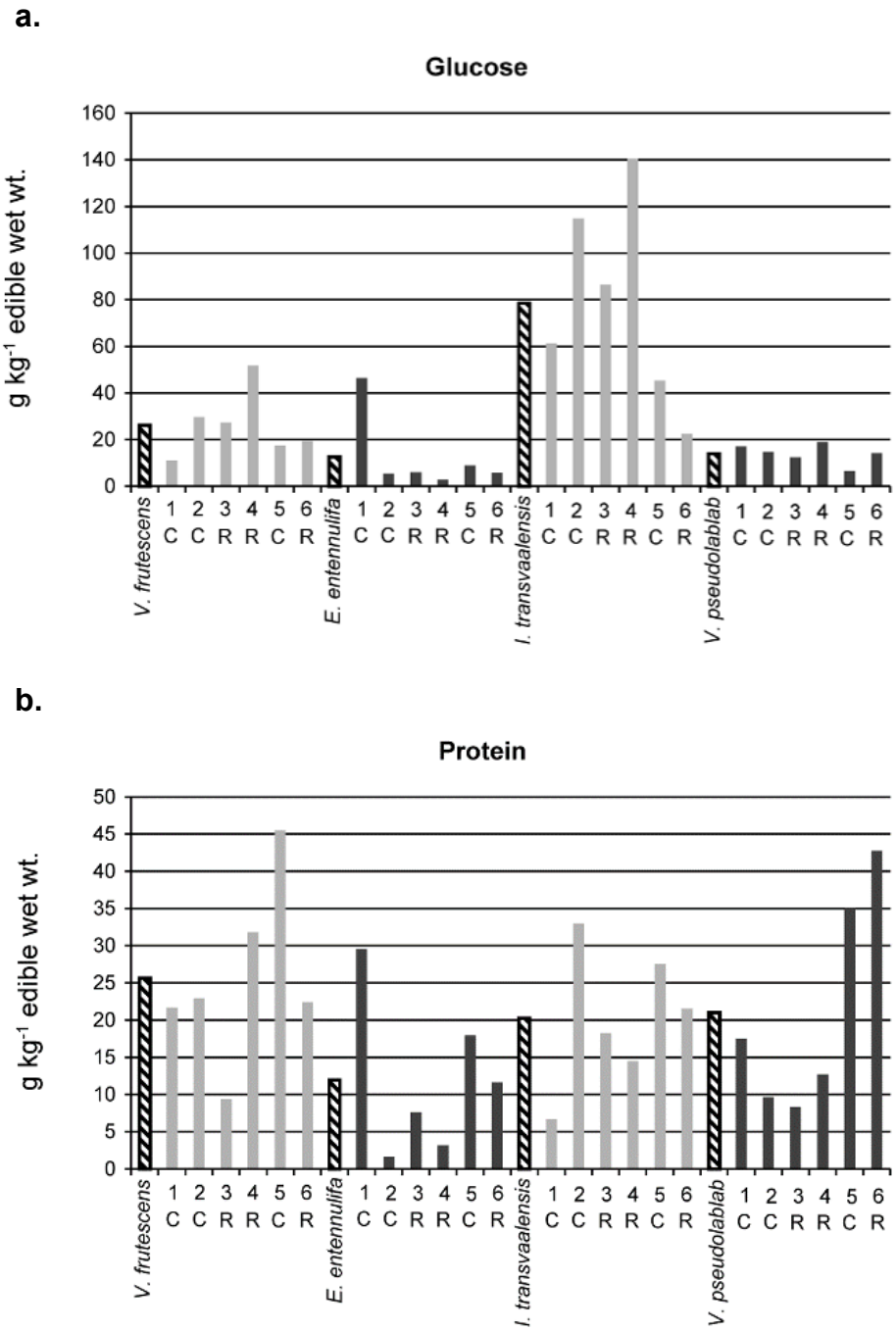


Fig 5. Glucose concentration (**a**) and protein (**b**) concentration in g kg-1 of edible wet weight as measured from starting meal samples for each run. Hatched black bars are averaged values for each tuber species. Concentrations have been corrected for dilution during the processing stage (simulated chewing) and are directly comparable. C cooked, R raw.

However, to fit our data within the context of previous work, we compared our glucose measurements to previous work on Hadza tuber nutrition (Schoeninger et al., 2001; Crittenden, 2009). Direct comparison is difficult because of different reporting schemes and analysis techniques. Despite this methodological challenge, we have attempted to make all work broadly comparable by converting values into grams per kilogram of whole wet weight, which we report in Supplementary Tables 2a-c. We found that Schoeninger et al. (2001) report the most similar values to our own data (Supplementary Tables 2a & b). Since we replicated the preprocessing techniques described by Schoeninger et al. (2001), this perhaps explains the relative match between these two studies, while other reports are consistently much higher in carbohydrate (starch plus simple sugars) values (Supplementary Table 2c) (Vincent, 1985; Crittenden, 2009). Protein values remain relatively consistent across studies.

3.3.4 Bioaccessibility

Nutritional bioaccessibility of wild tubers, presented here as the amount of glucose absorbed in the TIM-1 system relative to the input amount, was found to be quite variable in the small intestine (Figure 6). Tuber protein concentration was too low to reliably discriminate from the endogenous digestive enzyme matrix in the TIM-1 system and so absorption efficacy of nitrogen is not reported. Specimens with the highest absolute glucose absorption, *V. frutescens* and *I. transvaalensis*, had the lowest average percent glucose absorption (Table 4). This is not likely to be the result of any technical limitations of the TIM-1 system because of the continuous flow of high concentration digestive enzymes, mimicking physiological conditions (Minekus et al., 1995). Percent bioaccessibility of glucose averaged over the cooked and raw runs does indicate an effect of cooking for *V. frutescens* and *I. transvaalensis*. The absorption of glucose between raw and cooked *V. frutescens* differed by 10% (47.5% cooked versus 37.5% raw), whereas glucose absorption of raw *I. transvaalensis* is 9.1% greater than cooked (43.5% raw versus 34.4% cooked). On average, *E. entennulifa* and *V. pseudolablab* specimens did not show any appreciable difference in glucose absorption between raw and cooked runs. However, these results are limited owing to the low number of technical replicates and high degree of intraspecific variation. To resolve the issue of whether cooking affects bioaccessibility in a more statistically rigorous manner, we believe different methods should be used to allow for many more replicates and better isolation of variables.

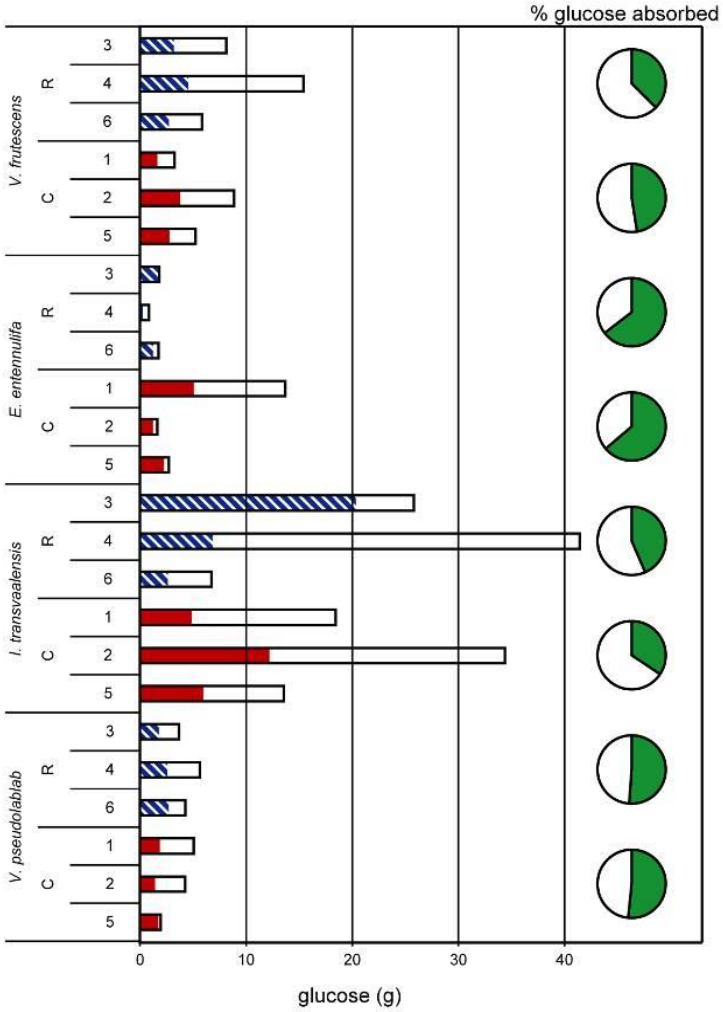


Fig 6. Summary of glucose absorption relative to input (g) and average bioaccessibility across all runs organized by specimen and state: cooked (C) or raw (R). Horizontal bars show the gram amount of input glucose (total hollow bar) and the gram amount absorbed (shaded area). These absolute values have been corrected for the dilution factor and are directly comparable to each other. Pie graphs show the percent of bioaccessible glucose (light shading) averaged over the three raw and three cooked runs for each tuber species.

Table 4 Summary of parameters to assess raw and cooked outcomes during TIM-1 digestion.[1]

Tuber	% bioaccessibility		loss coefficient[2]		hours until 95% max absorption[3]	
	Raw	**Cook**	**Raw**	**Cook**	**Raw**	**Cook**
V. frutescens	37.5 (±8)	47.5 (±5)	1.56	1.03	11 (4, 4, 3)	9 (3,3,3)
E. entennulifa	64.5 (±32)	63.7 (±24)	0.51	0.52	12 (3, 3, 6)	13 (4,3,6)
I. transvaalensis	43.5 (±31)	34.4 (±8)	0.98	1.61	15 (5, 5, 5)	11 (4, 4, 3)
V. pseudolablab	51.2 (±9)	51.6 (±30)	0.86	0.86	12 (4, 4, 4)	11 (3, 4, 4)

[1] According to three metrics- bioaccessibility, loss coefficient, and absorption timing- we predict that *V. frutescens* is best in cooked form, *I. transvaalensis* is best raw, and *E. entennulifa* and *V. pseudolablab* are not greatly affected by cooking. Values reported for bioaccessibility are mean ± standard deviation (in parentheses).

[2] Values greater than 1 indicate more glucose lost than absorbed (e.g. raw *V. frutescens* has 1.56 times glucose lost as is bioaccessible).

[3] Value is the sum total of hours for each run. In parentheses are the individual run hours for each of the three runs that were performed.

To explore the relationship between glucose absorption and glucose concentration of the starting meal, we plotted these two variables against each other for each species individually and tested for significance using the [cor.test] function in R. Outliers that produced an erroneous "two-point" correlation were removed for significance testing. We find that starting meal concentration significantly negatively correlates with the bioaccessibility of *V. frutescens* alone (cor.test, Spearman, $p = 0.033$), indicating that glucose concentration may be tied to other resistant or refractory carbohydrate. When we plotted this same relationship across all species, we see a clear significant negative relationship between glucose concentration of the meal (most likely deriving from starch and free D-glucose) and absorption of glucose (cor.test, Spearman, $p < 0.001$) (Figure 7). This means that absorption of glucose may be inhibited by the higher glucose content, such as found in starch, since these glucose polymers can resist digestion or overwhelm the enzyme activity in the small intestine. Therefore, a "glucose" rich tuber does not necessarily mean more glucose, proportionally, is directly available to the consumer. However, the undigested glucose is not entirely lost. Colonic microbiota preferentially use glucose based carbohydrate for fermentation into beneficial metabolic compounds, namely short chain fatty acids (SCFAs), which contribute to both host and microbial metabolism (Koropatkin et al., 2012). Because the TIM-1 model replicates only the upper digestive tract we are unable to assess the potential for the glucose in Hadza tubers to be digested by colonic bacteria.

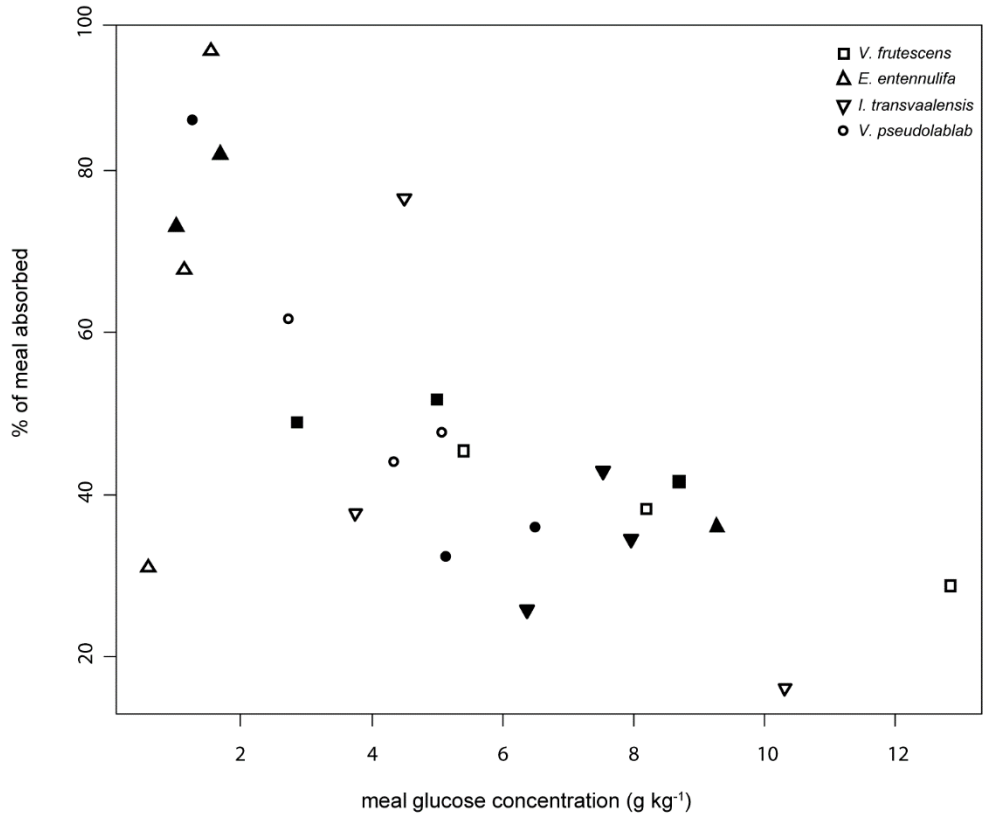


Fig 7. Plotted values of meal concentration and percent bioaccessibility of glucose for all specimens shows an overall negative relationship between these two variables, suggesting that more glucose dense specimens are more resistant to digestion. In contrast, lower glucose concentration is associated with higher glucose bioaccessibility, suggesting reduced concentration of refractory polysaccharides or higher concentration of free simple sugars, or perhaps a combination of both. Solid symbols are cooked specimens.

Finally, to fully assess the effect of brief roasting on the outcome of the digestion trials, we evaluated and summarized three different parameters taken from the results reported above. These parameters are 1) bioaccessibility, 2) loss coefficient, and 3) time until 95% of maximum absorption (Table 4). While bioaccessibility has been discussed previously and is relatively straight forward, loss coefficient is calculated from the difference between glucose accessibility of intake (percent of the meal glucose absorbed), and the glucose accessibility of recovery (percent of the dialysate, efflux, and residual glucose absorbed), divided back into the glucose accessibility of intake. The resulting value is a coefficient of the amount of glucose lost (for absorption) or inaccessible relative to the amount absorbed during digestion. This loss is attributed to refractory polysaccharides or clumping, making material inaccessible to the digestive enzymes in the model. Lastly, the time until 95% of maximum absorption was found from the kinetic data in which we have hourly absorption values over a six hour

period for all runs. Absorption typically peaked at two hours, however the rate of fall off noticeably varied, which gives an indication of resistance to digestion and length of time food is expected to remain in the gut. This is because a longer absorption curve over time means glucose is liberated and absorbed more slowly, potentially because of resistant starch and refractory fiber in the meal matrix. Therefore, a longer time until maximum absorption may indicate prolonged satiation, lower glycemic load, and use and motility of the entire small intestine, including the ileum, in digestion (a positive trait, implicated in helping maintain proper intestinal function). Based on these three parameters, a consumer interested in maximizing calories from glucose should roast their *V. frutescens*, since it provides more glucose and much less loss when cooked (Table 4). By the same token, the consumer should eat the *I. transvaalensis* raw, since it has greater glucose absorption and less loss when not cooked. We also observe the most protracted absorption period for raw *I. transvaalensis* (five hours for each of three runs) (Table 4).

3.3.5 Correlation of physical and nutritional properties

The physical properties of tubers described previously provide valuable contextual information to determine if these factors may relate to and be predictive of nutritional properties, including initial glucose concentration and final absorption. Kendall rank correlation coefficients were determined for correlations between physical properties: fresh weight, peel weight, fiber weight, and edible fraction; and nutritional properties: glucose concentration, protein concentration, glucose accessibility of the meal, and glucose accessibility of recovered meal after digestion (absorption plus efflux plus residual). Correlations were calculated only for those runs in which the meal dose comprised only one tuber specimen ($n = 8$, Supplementary Table 1). The calculation was also repeated with *I. transvaalensis* removed, since this was a considerable outlier, and interpretations are based only on significant or near significant tau (τ) values for both sets of calculations. Results are summarized in Tables 5 & 6 and Figures 8 & 9. Interestingly, fresh weight, fiber weight, peel weight, and edible weight all negatively correlate with the absolute value of both protein and glucose initial "meal" concentrations. Fresh weight in particular had the strongest negative associations, trending significant with regard to protein concentration. This suggests that larger and heavier tubers of these particular species may be dilute of caloric nutrients due to high water content.

Table 5. Kendall rank correlation coefficients (τ) determined by the [cor] function in R of tuber and nutritional properties for runs using single specimens (n = 8)[1].

nutritional properties	weight	fiber weight	peel weight	edible weight	edible fraction
glucose g/kg	-0.500	-0.400	-0.500	-0.286	0.286
percent of intake	-0.214	-0.400	-0.071	-0.286	0.143
percent of recovery	0.643**	0.546*	0.500	0.286	-0.429
glucose absorbed (abs)	-0.500	-0.400	-0.500	-0.286	0.286
protein g/kg	-0.571*	-0.255	-0.429	-0.500	0.071

*approaching significance ($p \sim 0.05$)

**significant ($p < 0.05$)

[1] Significance tested for individual correlations using the [cor.test] function in R with confidence level threshold set at 0.95.

```
z <- cor(y,x, use="complete.obs",method="kendall")
p <- cor.test(a,b, alternative="two.sided",method="kendall",conf.level=0.95)
```

Table 6. Kendall rank correlation coefficients (τ) determined by the [cor] function in R without the outlier species, *I transvaalensis*, using single specimens (n = 7)[1].

nutritional properties	weight	fiber weight	peel weight	edible weight	edible fraction
glucose g/kg	-0.333	-0.234	-0.333	-0.238	0.048
percent of intake	0.048	-0.238	0.238	-0.238	-0.143
percent of recovery	0.524	0.429	0.333	0.238	-0.238
glucose absorbed (abs)	-0.333	-0.238	-0.333	-0.238	0.048
protein g/kg	-0.619*	-0.143	-0.429	-0.524	-0.048

*approaching significance ($p \sim 0.05$)

[1] Significance tested for individual correlations using [cor.test] function in R with confidence level threshold set at 0.95.

```
z <- cor(b,a, use="complete.obs",method="kendall")
p <- cor.test(a,b, alternative="two.sided",method="kendall",conf.level=0.95)
```

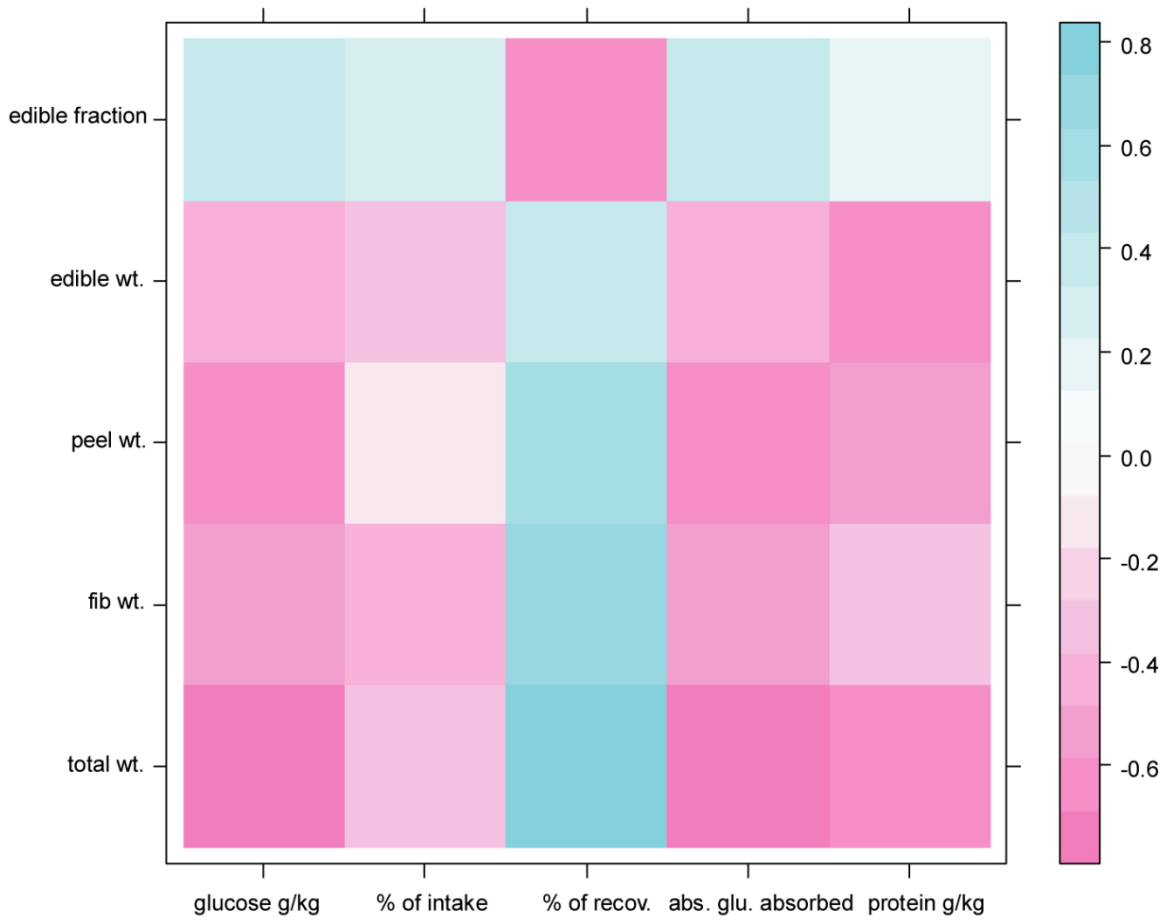


Fig 8. Kendall correlation heat map of single runs (n = 8), comparing physical properties (y-axis) to nutritional and digestion properties (x-axis). Glucose g/kg is the glucose concentration measured in the starting meal. Percent categories refer to the amount of glucose absorbed relative to the meal (intake), or recovery (recov.) glucose concentration. Teal shading indicates positive correlation while magenta shading indicates negative correlation. Abbreviations: abs. glu. absorbed (absolute glucose absorbed); % of recov. (% of recovery); fib. (fiber); wt. (weight).

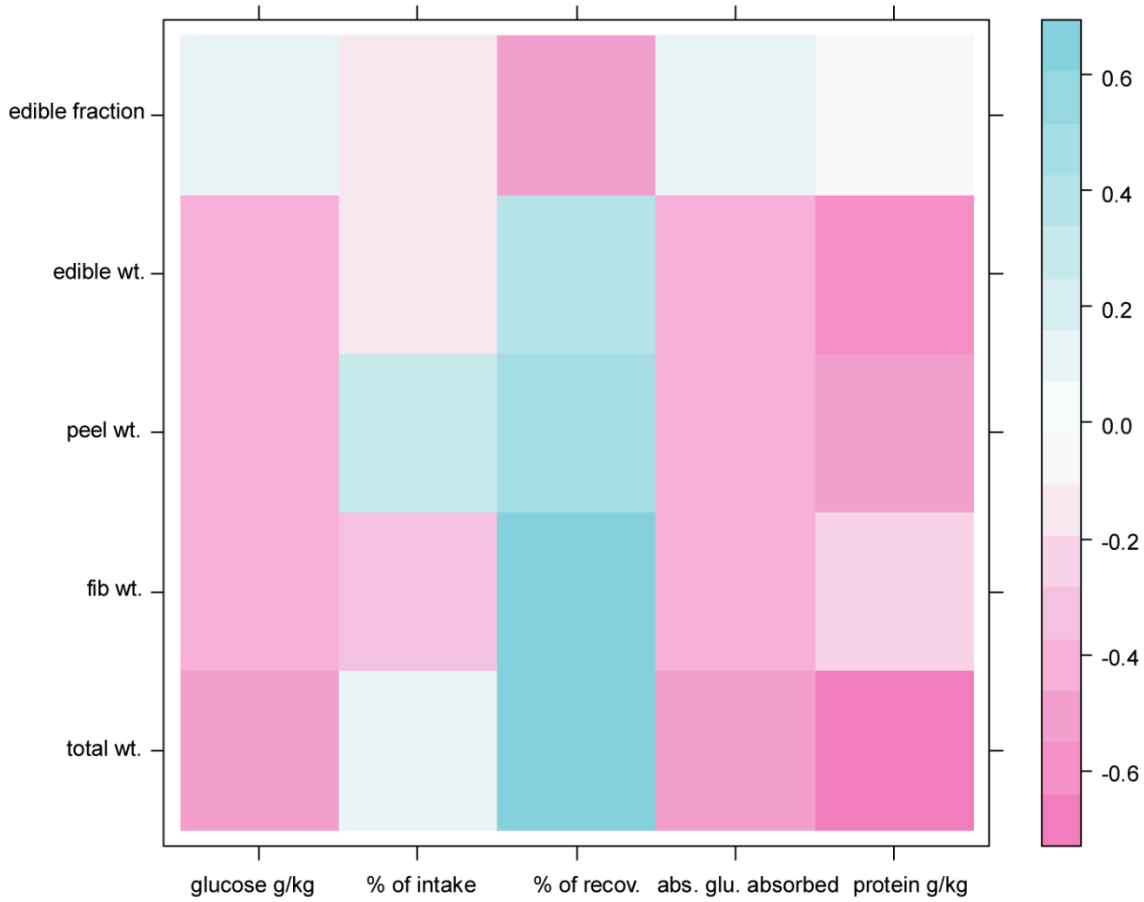


Fig 9. Kendall correlation heat map of singleton runs with *I. transvaalensis* outlier removed (n = 7), comparing physical properties (y-axis) to nutritional and digestion properties (x-axis). Glucose g/kg is the glucose concentration measured in the starting meal. Percent categories refer to the amount of glucose absorbed relative to the meal (intake), or recovery (recov.) glucose concentration. Teal shading indicates positive correlation while magenta shading indicates negative correlation. Abbreviations: abs. glu. absorbed (absolute glucose absorbed); % of recov. (% of recovery); fib. (fiber); wt. (weight).

3.3.6 Micronutrient analysis

Mineral concentration data for the consolidated meal aliquots and of the whole tuber specimens is reported in Table 7 and vitamin concentration data for the whole tuber specimens is reported in Table 8. For comparison we included published mineral and vitamin concentration data of cultivated and wild tuber species from the United States Department of Agriculture (USDA) National Nutrient Database for Standard Reference (http://ndb.nal.usda.gov/) and several other published sources (Finglas and Faulks, 1984; Bradbury and Holloway, 1988; Deshmukh and Rathod, 2013). We also included as reference the recommended dietary allowances (RDA) provided by the USDA based on the report from the Institute of Medicine's (IOM) Food and Nutrition Board (IOM, 1997, 1998, 2000, 2001, 2005, 2011). In the context of these comparisons, preliminary analyses suggest that Hadza tubers are rich in important minerals including calcium, potassium, magnesium, sulfur, iron, and manganese. When matched against RDA values, we see that Hadza tubers may be important contributors to daily dietary calcium, potassium, magnesium, iron, copper, manganese, iodine, chromium and molybdenum. The *penzepenze* "whole" tubers are especially rich in iron (22.0 mg $100g^{-1}$ fresh weight) and iodine (2.6 mg $100g^{-1}$), an interesting finding that we discuss later. Due to the starting residue content of calcium, potassium, and sodium salts that were part of the initial meal volume, however, the values for these elements were omitted for the Hadza tuber meal fractions in Table 7 (indicated as "NA"). Mineral and vitamin analysis for *penzepenze* are the average of five specimens reported per 100 grams of fresh weight. Vitamin values indicate that compared with domestic tubers, *penzepenze* is a relatively poor source of water soluble vitamins except, curiously, for B12, which is normally rare in plant resources and mainly acquired from animal derived foods. Vitamin B12 is critical for cellular metabolism, the nervous system, and brain function, but can only be synthesized by bacteria and archaea (Albert et al., 1980; Roth et al., 1996). The presence of B12 in plants stems from microbial biosynthesis and may be an indicator of microbial colonization of the plant tissue in this particular plant species. Nitrogen fixation is common in almost all of Fabaceae (legumes), supported by the presence of bacteria in root nodules (Bergersen, 1971). Earlier studies indicated that legume species grown with symbiotically fixed nitrogen are benefitted by the presence of cobalt in the soil, which is necessary for cobalamin (B12) production (Reisenauer, 1960; Evans and Kliewer, 1964). Vitamin B12 has been found to be concentrated in root nodules of legume plants (Reisenauer, 1960). Therefore, a mutualistic relationship may exist whereby bacteria colonize and facilitate nitrogen fixation for the plant, while the plant root system may help capture cobalt from the soil for microbial synthesis of essential B12 vitamins. Measurements of fat soluble vitamins in *penzepenze* are largely consistent with domestic varieties, which are a generally poor source of such nutrients, with the exception of vitamin A and carotenoids.

Table 7. Mineral analysis of Hadza tuber meal fractions and *penzepenze* with other wild and cultivated tubers[†]

Minerals	**Ca**	**P**	**Mg**	**Na**	**K**	**S**	**Fe**	**Cu**	**Zn**	**Mn**	**I**	**Cl**	**Se**	**Co**	**Cr**	**Mo**
RDA (mg/day)[1]	1000	700	420	1500	4700	ND	8	0.9	11	2.3	0.15	2300	0.055	ND	0.035	0.045
species	mg/100g fresh weight												µg/100g fresh weight			
Mak'alitako (*E. entennulifa*), raw	NA	6.6	63.3	NA	NA	5.2	1.5	0.2	0.2	0.1	ND	ND	0.6	2.9	49.7	1.5
Mak'alitako (*E. entennulifa*), roasted	NA	6.6	76.9	NA	NA	6.1	2.6	0.1	0.2	0.3	ND	ND	0.5	4.6	12.2	0.7
Shumuko (*V. pseudolablab*), raw	NA	23.4	107.8	NA	NA	9.3	2.3	0.1	0.2	0.1	ND	ND	0.9	1.8	7.7	2.5
Shumuko (*V. pseudolablab*), roasted	NA	13.0	90.2	NA	NA	3.4	2.1	0.1	0.2	0.1	ND	ND	0.4	1.7	8.9	1.9
//ekwa (*V. frutescens*), raw	NA	27.0	119.6	NA	NA	19.3	2.7	0.1	0.1	0.3	ND	ND	0.4	2.2	6.4	4.7
//ekwa (*V. frutescens*), roasted	NA	26.7	114.0	NA	NA	18.9	2.0	0.1	0.2	0.3	ND	ND	0.4	2.0	5.4	4.4
Panjuko (*I. transvaalensis*), raw	NA	45.8	55.8	NA	NA	26.4	1.4	0.1	0.4	1.1	ND	ND	1.1	1.6	7.5	30.0
Panjuko (*I. transvaalensis*), roasted	NA	21.9	31.1	NA	NA	14.1	1.1	0.1	0.2	0.5	ND	ND	0.5	0.9	5.9	16.9
Penzepenze (*Vigna* sp.), raw	210.0	37.0	82.0	8.9	590.0	15.0	22.0	0.1	0.4	0.8	2.6	110.0	0.5	13.0	300.0	41.0
Galya (*Brachystelma edulis*)[3]	93.0	28.7	37.3	1.9	83.3	ND	8.1	0.2	0.2	0.7	ND	ND	ND	ND	ND	ND
Kharpudi (*Ceropegia bulbosa var.*)[3]	96.2	34.1	32.6	2.7	88.1	ND	10.8	0.2	0.3	0.7	ND	ND	ND	ND	ND	ND
Haaman (*Ceropegia hirsuta*)[3]	103.6	36.4	36.9	2.5	106.5	ND	11.0	0.2	0.3	0.8	ND	ND	ND	ND	ND	ND
Sweet potato (*I. batatas*), raw[2]	29.0	51.0	26.0	52.0	260.0	13.0	0.5	0.2	0.6	0.1	ND	ND	ND	ND	ND	ND
Sweet potato (*I. batatas*), baked[2]	27.0	52.0	25.4	49.3	297.0	13.8	0.4	0.2	0.7	0.1	ND	ND	ND	ND	ND	ND
Taro (*C. esculenta*), raw[2]	32.0	70.0	115.0	1.8	448.0	8.5	0.4	0.2	3.8	0.4	ND	ND	ND	ND	ND	ND
Taro (*C. esculenta*), baked[2]	31.1	74.5	115.3	1.6	442.0	8.1	0.3	0.2	3.9	0.4	ND	ND	ND	ND	ND	ND
Giant taro (*A. macrorrhiza*), raw[2]	38.0	44.0	52.0	30.0	267.0	12.0	0.8	0.1	1.6	0.6	ND	ND	ND	ND	ND	ND
Giant taro (*A. macrorrhiza*),cooked[2]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Yam (*D. alata*), raw[2]	8.2	38.0	17.0	3.3	318.0	12.0	0.6	0.2	0.4	0.0	ND	ND	ND	ND	ND	ND
Yam (*D. alata*), baked[2]	7.7	35.5	15.9	2.5	295.0	11.9	0.5	0.2	0.4	0.0	ND	ND	ND	ND	ND	ND
Cassava (*M. esculenta*), raw[2]	20.0	46.0	30.0	7.2	302.0	6.4	0.2	0.1	0.5	0.1	ND	ND	ND	ND	ND	ND
Cassava (*M. esculenta*), baked[2]	19.1	42.4	12.2	6.3	281.0	6.2	0.2	0.1	0.6	0.1	ND	ND	ND	ND	ND	ND
Potato (*S. tuberosum*), raw[3]	5.6	35.0	14.1	10.7	320.0	ND	0.3	0.1	0.2	ND	ND	ND	ND	ND	ND	ND
Potato (*S. tuberosum*), roasted[3]	9.2	55.0	24.6	9.2	565.0	ND	0.6	0.1	0.4	ND	ND	ND	ND	ND	ND	ND

[†] ND = no data; NA = not applicable

[1] Dietary Reference Intakes: RDA and AI for Vitamins and Elements based on male 19-30 yrs., accessed from [online] fnic.nal.usda.gov/dietary-guidance/dietary-reference-intakes/dri-tables; [2] Bradbury and Holloway, 1988; [3] Finglas and Faulks, 1984; [4] Deshmukh and Rathod, 2013

Table 8. Vitamin analysis of Hadza tuber, *penzepenze*, and other cultivars[†]

Vitamins	**B1**	**B2**	**B3**	**pantothenic acid**	**B6**	**C**	**E**	**biotin**	**folate**	**B12**	**K1**	**A**	**D**	**carotenoids**
RDA (mg/day)[1]	1.2	1.3	16	5	1.3	90	15	0.03	0.4	0.0024	0.12	0.9*	0.015	NA
species	mg/100g fresh weight							μg/100g fresh weight						
Penzepenze (*Vigna sp.*), raw	0.007	0.03	0.25	1.2	0.048	1.2	0.2	2.8	9.5	0.97	< 0.4	< 0.3	< 50	9.6
Sweet potato (*I. batatas*), raw	0.086[2]	0.031[2]	0.92[2]	ND	0.209[3]	24[2]	0.26[3]	ND	11[3]	0	1.8[3]	11*[2]	0	NA
Taro (*C. esculenta*), raw	0.032[2]	0.025[2]	0.95[2]	ND	0.283[3]	15[2]	2.38[3]	ND	22[3]	0[3]	1.0[3]	7*[2]	0[3]	NA
Giant taro (*A. macrorrhiza*), raw	0.021[2]	0.018[2]	0.94[2]	ND	ND	17[2]	ND	ND	ND	ND	ND	0*[2]	ND	NA
Yam (*D. alata*), raw	0.047[2]	0.03[2]	0.82[2]	ND	0.293[3]	28[2]	0.35[3]	ND	23[3]	0[3]	2.3[3]	18*[2]	0[3]	NA
Cassava (*M. esculenta*), raw	0.05[2]	0.04[2]	0.67[2]	ND	0.088[3]	15[2]	0.19[3]	ND	27[3]	0[3]	1.9[3]	4.9*[3]	0[3]	NA
Potato (*S. tuberosum*), raw	0.2[4]	0.02[4]	0.4[4]	ND	0.24[3]	16[4]	ND	ND	25[4]	0[3]	ND	0*[3]	0[3]	NA

[†] ND = no data; NA = not applicable

* includes both vitamin A and carotenoids

[1] Dietary Reference Intakes: RDA and AI for Vitamins and Elements based on male 19-30 yrs., accessed from [online] fnic.nal.usda.gov/dietary-guidance/dietary-reference-intakes/dri-tables; [2] Bradbury and Holloway, 1988; [3] USDA nutrition tool, accessed from [online] http://ndb.nal.usda.gov/; [4] Finglas and Faulks, 1984

3.4 Discussion and conclusions

The results of our digestion experiments on raw and briefly roasted Hadza tubers demonstrate that bioaccessibility plays a significant role in the nutritional value of foods. For the four Hadza tubers we tested, we found that glucose absorbed relative to total glucose is considerably limited in the *in-vitro* GI environment. This is in part due to motility of the fibrous parenchyma, which tended to clump and limit exposure to gastric and pancreatic enzymes, and to the presence of indigestible fiber or refractory carbohydrates. Previous nutritional studies that analyzed fiber content of Hadza tubers found a relatively high percentage of fiber, between approximately 10% and 30%, in the form of soluble (pectin) and insoluble (cellulose, hemicellulose, and lignin) fiber (Vincent, 1985; Schoeninger et al., 2001; Crittenden, 2009). Fiber as well as other nutrients, even those that are digestible, can form complexes with non-structural carbohydrates, proteins or fats to resist the activities of digestive enzymes and diminish caloric returns. For this reason, discovering the bioaccessible components of Hadza tubers, and even more, their site of activity in the upper or lower gut, is essential to our understanding and interpretation of the overall nutritional contribution of these foods to human foragers. However, this study does not take into account the cost of chewing, and there is good evidence that the oral comminution phase of digestion is in fact a significant bottleneck to obtaining edible matter from Hadza tubers. Indeed, chewing efficiency is a known aspect of tuber digestibility that measurably increases as a result of cooking (Dominy et al., 2008).

In this experiment, we found that absorption was limited to anywhere between 35% and 65% of total glucose availability in the edible fraction of four Hadza tubers. Factoring this fraction in to the original tuber glucose concentration produces very low estimates of actual glucose contribution per kilogram of whole tuber. However, Hadza tubers are a plentiful food resource (Vincent, 1985; Marlowe and Berbesque, 2009), available year round, and routinely exploited by Hadza women, who have been observed to procure an average of 6.1kg of tuber per foraging foray (McDowell, 1981; Vincent, 1985). Women frequently make more than one foray per day, once in the morning and then again in the early evening. Observations of much greater tuber acquisition (20+ kilos per woman) are not unheard of (Vincent, 1985). While some forage is consumed away from camp, the result of snacking, the majority is brought back to camp and shared with other members that did not participate in foraging; usually young children, men, and older adults. Even with snacking and sharing, we may suppose that an adult Hadza woman acquires approximately two to four kilograms of *V. frutescens* tuber per day (Vincent, 1985), which based on our measurements of glucose accessibility alone, could satisfy up to roughly one fifth to one quarter of daily calories based on a total energy expenditure of 1877 ± 364 kcal per day (Pontzer et al., 2012). This estimate does not include calories that may be obtained from protein, fat, or non-glucose based carbohydrate content of *V. frutescens*. In addition, it does not take into account the

energy obtained from fermentation by the gut microbiota in the large intestine, which leads to the production of SCFAs. These are also potential energy substrates for the host (Cummings et al., 1987; Louis et al., 2007; Flint et al., 2008). We therefore expect the true caloric values of these tubers to be even greater than our estimation based on upper GI digestion alone.

Interestingly, there was greater variation seen within species during the initial individual runs than when specimens of each species were pooled. However, the high intraspecific variation and inconsistent results with homogenized runs indicates that we have either not captured a "representative" sample, or, that a real representative sample is impossible, as we believe to be true. For wild foods in particular, there are a number of uncontrolled variables such as yearly rainfall patterns, plant location, plant age, soil composition, and plant health, to name a few. Although we cannot emphasize statistically conclusive results about the effect of cooking, we believe the consistent pattern of high intraspecific variation observed in all studies of Hadza tubers brings to light some very interesting implications about human forager reliance on plant food resources. This suggests that the acquisition of glucose hinges not on roasting, but on how adept the forager is at selecting the individual plant that bears the highest quality tuber. Foraging requires keen botanical knowledge of the best plants to target, and geographic awareness of locations that enable plant growth. In addition, a foraging subsistence likely induces physiological adaptations that selectively increase metabolic efficiency in a resource limiting or unpredictable environment. Such traits include a reduction of metabolic rate (Pontzer et al., 2012) or specialization of gut microbiota to harvest and produce energy for the host (Cummings et al., 1987; Louis et al., 2007). We note that considerable loss occurred during digestion because of clumping and high fiber content preventing exposure to digestive enzymes and absorption, and much of the tuber pulp may pass the ileocaecal valve into the large intestine where fermentation with gut microbiota can take place. We stress the importance of activating the whole gut in digestion and that resistant carbohydrate material, such as tubers, can help sustain the health of the colonic ecosystem (Hijova and Chmelarova, 2007; Flint et al., 2008; Kau et al., 2011; Koropatkin et al., 2012). Indeed, the colon can provide additional metabolic products to the host in the form of SCFAs when fed carbohydrates, and it is likely that Hadza possess a unique suite of microbiota specifically adapted to deal with a vast array of refractory and resistant fiber (Schnorr et al., 2014).

Different species of tuber harbor unique nutritional qualities that are not always related to caloric value. Water is a critical human requirement, and certainly so during the dry season in the arid East African climate. Highly edible wild tubers, both in terms of edible fraction and accessible glucose, may actually be more important for their moisture rather than caloric contribution. Our results and previous work demonstrate that *V. pseudolablab* and *E. entennulifa*, two of the regularly consumed Hadza tubers, contain low glucose concentrations and are approximately 70% and 90% moisture (Schoeninger et al., 2001; Crittenden, 2009). Taxonomically and physically similar tuber species were likely in existence in savanna-mosaic landscapes for a substantial portion of human evolution, and may have facilitated occupation of arid climates and expansion away from permanent water features such

as oceans, lakes and rivers (Schoeninger and Bunn, 2009). We found that fresh weight correlated negatively with glucose and protein concentration. If indeed larger tubers do retain more water, then this dilution effect can be problematic for nutritional reporting depending on whether values are expressed on a dry or wet weight basis. Expressing values on a dry weight basis, while remaining the common convention in the literature, was not only impossible for our study, but may also alter the perceived value of a resource. When the nutritional profile of a food is reported relative to the total dry weight, calories alone become the "currency" rather than the entire edible wet weight (i.e. nutrients are reported on a condensed weight scale). While reporting dry weight remains valuable, particularly for comparative studies, it is also important to consider the entire wet weight material for studies modeling human food consumption because the water content inflates the amount of mass consumed relative to nutrition, meaning a consumer may get physically full from the amount of material eaten, even when fewer calories are consumed. Using fresh weight as the basis of measure is important in the context of modeling forager food intake because tubers are consumed fresh, and so consumption may be physically limited on a fresh-weight basis due to volume of the tuber and capacity of the gut.

Non-caloric nutrients, called micronutrients, are necessary for metabolic and immune function, rendering these vitamins and minerals critical for survival. Micronutrient deficiency, or malnutrition, can lead to a number of diseases due to metabolic dysregulation or increased susceptibility to infection; the recognition and understanding of which has only been thoroughly articulated in the last century (Keusch, 2003). Therefore, by knowing human nutritional requirements, we can extrapolate this information to infer whether a particular environment is sustainable for a human forager, or to what extent limitations are overcome through behavioral adaptations. Since there has been very little work to date on the plant micronutrient landscape in human forager/forager-horticulturalist environments, our preliminary assessment of the vitamin and mineral content of four tuber meal fractions and one whole tuber offers an initial assessment for how Hadza tubers contribute to nutritional homeostasis in an East African savanna-mosaic environment. The mineral content is quite favorable for certain elements essential to metabolism and functioning of the immune and nervous system, such as magnesium, iron, potassium, calcium, and iodine. Iodine concentration in *penzepenze* is especially interesting since inland environments are often considered iodine deficient, to the extent that many modern foods are fortified with iodine. In fact, some of the most important tropical tuber crop cultivars, such as sweet potato (*I. batatas*) and cassava (*M. esculenta*), are notably goitrogenic, meaning they prevent uptake of iodine, causing suppression and enlargement of the thyroid gland (Johns, 1996; Venturi and Begin, 2010). Iodine is essential for proper thyroid function and acts directly on brain growth by helping regulate thyroid hormone production and signaling development in a dose-dependent manner (Venturi and Begin, 2010). Most dietary iodine comes from aquatic or marine resources, an observation favoring the idea (and mounting evidence) that aquatic environments were a central presence in human evolution (Braun et al., 2010; Colonese et al., 2011; Archer et al., 2014). While we do not have any information on the iodine restriction potential of *penzepenze*, our preliminary

analysis of the iodine concentration is interesting and merits further investigation. Given the concurrent enrichment of iron in all Hadza tubers, especially *penzepenze*, which is twice that reported for wild tubers in India (Deshmukh and Rathod, 2013) and nearly three-fold greater than the RDA for iron, we posit that volcanic and Rift Valley geological activity over time may have enriched soils with mineral deposits that are in turn harvested by the plants of that region. Vitamin analysis of *penzepenze* (the only Hadza tuber for which vitamin results are reported with confidence) again shows an unexpectedly high concentration of vitamin B12, which is not common in plant food resources. Animals require B12 for metabolism and proper functioning of the central nervous system, and must obtain it from the diet or from microbial biosynthesis. The curious presence of this water-soluble vitamin in *penzepenze* could be an indication of rich microbial activity in the soil, setting up a mutualistic relation between the soil microbiota and the plants for exchange of nutrients, particularly for Fabaceae plants (Ramirez-Puebla et al., 2013). In this way, B12 enrichment may occur for Hadza tubers, placing increasing importance on tuber nutritional contribution in the form of micronutrients, rather than macronutrients.

Control of fire and cooking is considered a watershed moment in human evolutionary history because of its potential to greatly increase the nutritional value of foraged foods and dramatically extend the breadth of edible resources (Stahl, 1984; Laden and Wrangham, 2005). Cooking is widely regarded to improve the nutritional value of foods through a number of physical and chemical changes that enable more efficient digestion (Stahl, 1984; Johns, 1996). Therefore, we would expect that light roasting, as practiced by Hadza on wild tubers, would enable more efficient acquisition of calories. Our results indicate that cooking had a range of effects on glucose absorption depending on both specimen and species, however, these results lack statistical power given the low number of replicates. Most interesting is that cooking, on average, improved glucose absorption for *V. frutescens*, which is the species most favored by Hadza and almost always roasted (Crittenden, 2009; Crittenden, in press). Cooking had no effect on glucose absorption for *E. entennulifa* and *V. pseudolablab*, and curiously negatively impacted absorption for *I. transvaalensis*, which is typically consumed raw. Therefore, we now have empirical data that aligns with observed behavior of different tuber roasting practices among the Hadza. Since *E. entennulifa* and *V. pseudolablab* are large high moisture tubers with low glucose concentration by fresh weight, brief roasting likely does not gelatinize interior starches. Furthermore, low concentrations of native starch are probably efficiently hydrolyzed by salivary and pancreatic amylase. The negative impact of brief roasting on glucose absorption of *I. transvaalensis* tubers may stem from the high degree of clumping that occurred for cooked rather than raw specimens, reducing their exposure to digestive enzymes in the model.

While roasting had variable effective outcomes on tuber digestibility, one general effect of brief roasting is that it not only softens the parenchymatous tissue, but in addition, it dramatically softens the cortex of some species of Hadza tubers, particularly *penzepenze*, hastening tuber peeling, cutting, and fiber extraction (Dominy et al., 2008). Therefore, softening for ease of peeling and

chewing is a likely key motivator for the brief roasting behavior (Woodburn, 1966; Tomita, 1968). In our experience with peeling and simulated chewing of raw and roasted Hadza tubers, brief roasting greatly increased manual ease of access to the internal and edible tuber pulp. Therefore, we hypothesize that brief roasting has universal appeal for the changes induced to mechanical and physical properties and limited improvement in the direct biological value of Hadza tubers. However, this study did not take into account the energetic cost of chewing. There is good evidence that the oral comminution phase of digestion is in fact a significant bottleneck to the overall bioaccessibility of Hadza tubers. Indeed, the net caloric gain for a human consumer would certainly be higher for roasted tubers due to a measurable increase in chewing efficiency (Dominy et al., 2008; Zink et al., 2014). Compared to modern domestic cultivars such as yam, potato, cassava, sweet potato, and taro, all of which except sweet potato must be fully cooked for human consumption, Hadza tubers have relatively low glucose and carbohydrate concentrations and can be consumed raw as well as lightly roasted, much like the high moisture, low starch Jicama (*Pachyrhizus erosus*) (Finglas and Faulks, 1984; Vincent, 1985; Bradbury and Holloway, 1988; Schoeninger et al., 2001; Stevenson et al., 2007), a finding supported by multiple independent measurements. The relatively low density of starches and high concentration of simple sugars and moisture may make cooking of little relevance for effective digestion, but critical to physical access and mastication, especially for young children (Schoeninger et al., 2001; Crittenden, 2009). However, Hadza tubers may be uniquely deplete in carbohydrates, even among other wild roots and tubers that are targeted by other foraging populations. The composition of wild yams (*Dioscorea* spp.) from central Africa analyzed by Hladik et al. (1984) included between 54.2 and 79.8 percent of dry weight of starch and soluble sugars combined (a min - max of 146 - 324 g kg^{-1} wet weight respectively). Analysis of edible plant foods in South Africa, however, yielded many comparable carbohydrate values to our current assessment of the edible portions of Hadza tubers (Youngblood, 2003). In addition, of the five edible species selected for laboratory analysis in Youngblood (2003), none exhibited cyanogenetic glycoside poisons that are typical of many tropical bulbous plants (such as cassava), and several species are known to be targeted by children and consumed raw or slightly roasted. The disparity in these findings clearly illustrates that wild plant food nutrition is highly variable by region, and that nutritional data from a single region (even if comprehensive), should not be used to generalize interpretations about plant food contribution to the human diet.

The result of 24 *in-vitro* digestion trials with four species of tuber regularly consumed by Hadza foragers indicates that anywhere from one third to more than half of the available glucose, namely from simple sugars and starch, is not absorbed. These results, coupled with the overall finding that Hadza

tubers have low edible fractions, suggest that while wild tubers are an undeniably important resource, their caloric contribution from glucose is significantly hampered by resistant carbohydrate and fiber. Removal of physical barriers such as the peel and inedible fiber is greatly expedited through the use of roasting, whereas the absorption of glucose in tubers was not appreciably affected by roasting. We conclude that food processing, and cooking in particular, can be useful for reasons that may not necessarily involve nutrition or bioaccessibility directly. Instead, in the case of the wild tubers roasted and consumed by Hadza, ease of peeling, softening, separation of fibers, bulking and gut transit time seem to play a greater role in affecting glucose absorption through digestion. Future work is needed to fully establish the specific role of cooking, vitamin and mineral concentrations, and the digestibility of such micronutrients and proteins. Preliminary analyses indicate that these nutritional components especially may contribute to the overall resource value of Hadza tubers.

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