Nutritional contribution of plant foods to human diet in evolution

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ADDENDUM

Summary

Cooking is an important technology for its capacity to increase food digestibility, potentially playing a significant role in human evolution as a means to increase nutritional density of food. However, the contextual impetus for cooking is less well understood. One mode of cooking used by modern humans is to roast food directly in an open flame fire for a wide range of times. This technique may be a behavioral analogue to that of early human ancestors. Here, we provide the first experimental data on starch gelatinization patterns of USOs and starchy fruit as a result of brief fire roasting. We employed a count-based method using light microscopy and a semi-quantitative photometric strategy to observe the kinetic process of gelatinization. We find that brief, five-minute roasting over an open fire results in low levels of starch gelatinization in the parenchymal tissue, which persists even after longer roasting durations, exceeding ten minutes. However, at fifteen and twenty minutes, the majority of starch throughout each specimen is sufficiently gelatinized to enable alpha-amylase activity. These findings suggest that instances of very brief roasting, up to ten minutes, may not necessarily benefit a consumer through changes to starch structure and susceptibility to alpha-amylase activity alone.

With an initial understanding of the role of brief roasting on starch gelatinization, we then turned to the question of nutritional bioaccessibility of raw and briefly roasted wild tubers to a human consumer. 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 elucidated 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.

To continue to evaluate the efficacy of digestion of fibrous plant foods by human consumers, we targeted the gut microbiota to explore its composition and function as an essential factor in human

digestion. Human gut microbiota directly influences health and provides an extra means of adaptive potential to different lifestyles. To explore variation in gut microbiota and to understand how these bacteria may have co-evolved with humans, here we present the first investigation of phylogenetic diversity and metabolite production of the gut microbiota from a community of human hunter-gatherers, the Hadza of Tanzania. We show that the Hadza have higher levels of microbial richness and biodiversity than Italian urban controls. Further comparisons with two rural farming African groups illustrate other features unique to Hadza that can be linked to a foraging lifestyle. These include absence of *Bifidobacterium* and differences in microbial composition between the sexes that probably reflect sexual division of labor. Furthermore, enrichment in *Prevotella*, *Treponema*, and unclassified Bacteroidetes, as well as a peculiar arrangement of Clostridiales taxa, may enhance the Hadza's ability to digest and extract valuable nutrition from fibrous plant foods.

Samenvatting

Koken speelde een belangrijke rol in de evolutie van de mens, doordat het de verteerbaarheid van voedingsmiddelen vergroot en de nutriëntendichtheid ervan kan verhogen. We begrijpen echter nog steeds weinig van de specifieke context die aanleiding gaf tot het verhitten van voedsel. Een manier van voedselbereiding die door moderne mensen gebruikt wordt, is het roosteren van voedsel, direct in de vlammen van een open vuur, gedurende kortere of langere tijd, wellicht een goede analogie voor het kookgedrag van vroegere mensachtigen. Deze studie presenteert de eerste experimenteel verkregen data met betrekking tot patronen van zetmeel gelatinisering in USO's (Underground Storage Organs: plantenwortels en knollen) en zetmeelrijke vruchten als gevolg van kort roosteren boven een open vuur. Er werd gebruik gemaakt van tellingen met behulp van microscopie en een semi-kwantitatieve fotometrische strategie om de kinetische processen van gelatinisering te observeren. Kort (vijf minuten lang) roosteren boven een open vuur blijkt te resulteren in lage niveaus van zetmeel gelatinisering van het parenchymatisch weefsel, niveaus die ook na langer roosteren, tot meer dan tien minuten, laag blijven. Bij 15 en 20 minuten roosteren blijkt het merendeel van het zetmeel in elk specimen voldoende gegelatiniseerd om alpha-amylase activiteit mogelijk te maken. Deze bevindingen suggereren dat kort roosteren (tot tien minuten) een consument geen voordeel zal opleveren in termen van veranderingen in de zetmeelstructuur en toegankelijkheid voor alpha-amylase activiteit alleen.

Met deze basiskennis van de rol van kort roosteren in gelatinisering van zetmeel is vervolgens gekeken naar de nutritionele bioaccessibiliteit van rauwe en kort geroosterde wilde knollen voor de menselijke consument. Bioaccessibiliteit is een nuttige maat voor het beoordelen van de biologische waarde van een bepaalde nutriënt in voedsel, met name in knollen en dergelijke. De wilde knollen die door de Hadza jagers-verzamelaars in Tanzania gegeten worden zijn interessant omdat ze niet giftig zijn, rauw of kort geroosterd geconsumeerd worden en nogal wat fysieke "barrières" met zich meebrengen voor de consument. In dit onderzoek probeerden we inzicht te krijgen in de biologische waarde van de Hadza knollen door het meten van de opname van glucose door middel van in-vitro digestie. Wij kwantificeerden verteerbaarheid op basis van data van 24 experimenten op vier soorten door de Hadza gebruikte knollen, gebruikmakend van een dynamisch in-vitro model dat digestie in maag en dunne darm nabootst. Analyse van de glucose in de "input"-maaltijd en het "output"-dialysaat leverde de toegankelijke glucose fractie op. We analyseerden ook eiwit, vitaminen en mineralen-samenstelling van hele knollen en gefractioneerde maaltijden. Bioaccessibiliteit van glucose verschilt per soort. Als we het effect van kauwen constant houden, blijkt kort roosteren een verwaarloosbaar effect te hebben, maar de grote variatie binnen één soort beperkt de draagwijdte van deze conclusies. In het algemeen blijken de door de Hadza geconsumeerde knollen zeer resistent tegen digestie, met gemiddeld een- tot twee-derde van de glucose opgenomen. De fractie geabsorbeerde versus input

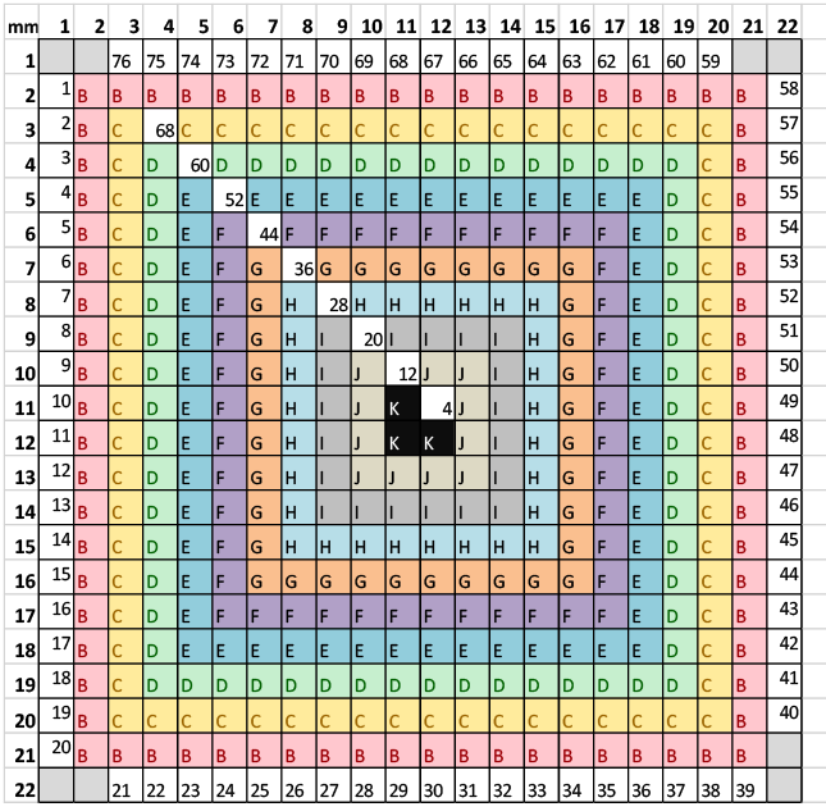
glucose correleerde negatief met de totale glucose concentratie (in vrije en gepolymeriseerde staat) in de knollen. Roosteren levert wellicht andere voordelen op, zoals gemakkelijker pellen en kauwen om het eetbare parenchymweefsel te extraheren. Een belangrijke factor in glucosevoorziening is de kwaliteit van de knol, waar de kundigheid van de individuele verzamelaar een belangrijke rol in speelt. Andere nutriënten-analyses leverden onverwacht hoge waarden voor eiwitten, ijzer en jodium, hetgeen aangeeft dat knollen wellicht niet alleen vanwege hun calorieën waardevolle voedselbronnen zijn.

Voor verdere evaluatie van de efficiëntie waarmee menselijke consumenten vezelrijk plantaardig voedsel verteren hebben we ons gericht op de microbiota van het maag-darmkanaal, ter verkenning van compositie en functie als een wezenlijke factor in de menselijke spijsvertering. De microbiota van de menselijke darm beïnvloeden rechtstreeks de menselijke gezondheid en vormen een extra aanpassingspotentieel aan verschillende leefwijzen. Als een verkennende studie van de variatie van microbiota in het maag-darmkanaal en om te begrijpen hoe de co-evolutie van deze bacteriën met mensen verlopen kan zijn, presenteren we hier het eerste onderzoek van de fylogenetische diversiteit en de metabolieten-productie van de maag-darm microbiota van een groep jagers-verzamelaars, de bovengenoemde Hadza uit Tanzania. De Hadza blijken gekenmerkt door een hogere microbiële rijkdom en biodiversiteit dan een Italiaanse stedelijke controlegroep. Verdere vergelijkingen met twee agrarische rurale groepen in Afrika illustreren andere kenmerken die uniek zijn voor de Hadza en aan een leefwijze als jager-verzamelaar gekoppeld kunnen worden: o.a. afwezigheid van *Bifidobaterium* en geslachtsgebonden verschillen in microbiële compositie die waarschijnlijk samenhangen met een geslachtsgebonden arbeidsverdeling. Bovendien zou de verrijking in *Prevotella*, *Treponema* en ongeclassificeerde Bacteroidetes soorten alsmede een ongebruikelijke samenstelling van Clostridiales taxa wellicht ook het vermogen van de Hadza kunnen vergroten om vezelrijk plantenvoedsel te verteren en er waardevolle voedingsstoffen uit te halen.

Appendix A

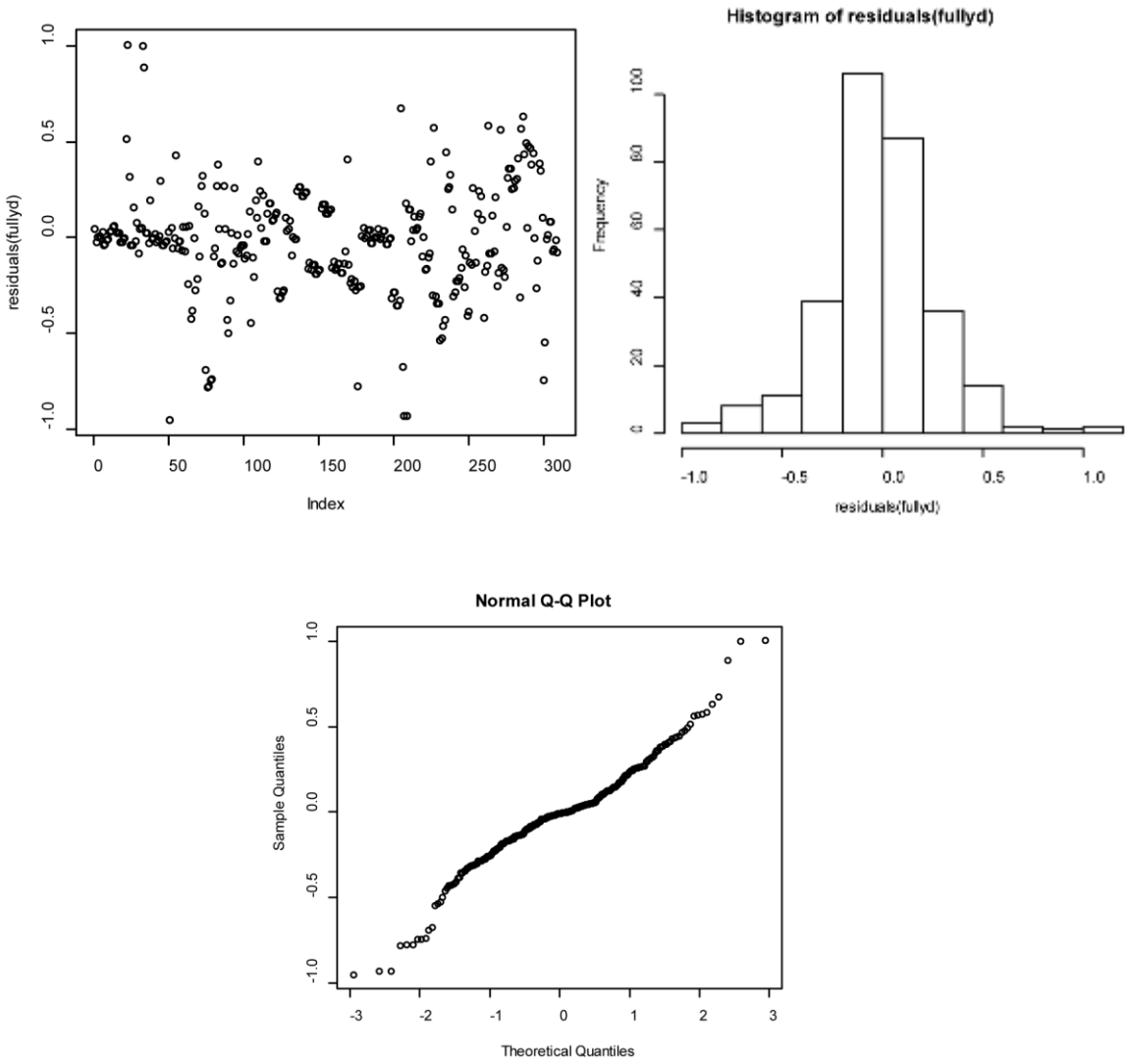
Chapter 2 supplementary information

Supplementary Figures



mm	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1			76	75	74	73	72	71	70	69	68	67	66	65	64	63	62	61	60	59		
2	1	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	58
3	2	B	C	68	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	B	57
4	3	B	C	D	60	D	D	D	D	D	D	D	D	D	D	D	D	D	D	C	B	56
5	4	B	C	D	E	52	E	E	E	E	E	E	E	E	E	E	E	E	D	C	B	55
6	5	B	C	D	E	F	44	F	F	F	F	F	F	F	F	F	F	E	D	C	B	54
7	6	B	C	D	E	F	G	36	G	G	G	G	G	G	G	G	F	E	D	C	B	53
8	7	B	C	D	E	F	G	H	28	H	H	H	H	H	H	G	F	E	D	C	B	52
9	8	B	C	D	E	F	G	H	I	20	I	I	I	I	H	G	F	E	D	C	B	51
10	9	B	C	D	E	F	G	H	I	J	12	J	J	I	H	G	F	E	D	C	B	50
11	10	B	C	D	E	F	G	H	I	J	K	4	J	I	H	G	F	E	D	C	B	49
12	11	B	C	D	E	F	G	H	I	J	K	K	J	I	H	G	F	E	D	C	B	48
13	12	B	C	D	E	F	G	H	I	J	J	J	J	I	H	G	F	E	D	C	B	47
14	13	B	C	D	E	F	G	H	I	I	I	I	I	I	H	G	F	E	D	C	B	46
15	14	B	C	D	E	F	G	H	H	H	H	H	H	H	H	G	F	E	D	C	B	45
16	15	B	C	D	E	F	G	G	G	G	G	G	G	G	G	G	F	E	D	C	B	44
17	16	B	C	D	E	F	F	F	F	F	F	F	F	F	F	F	F	E	D	C	B	43
18	17	B	C	D	E	E	E	E	E	E	E	E	E	E	E	E	E	E	D	C	B	42
19	18	B	C	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	D	C	B	41
20	19	B	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	B	40
21	20	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	
22			21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	

Supplementary Figure 1. Schematic showing the 22 mm cover slip area and the ten concentric squares that represent the area analyzed. The outer 2 millimeter edge was excluded. The ten concentric squares of the cover-slip area were systematically analyzed using a random coordinate generator to select a one millimeter square area for observation per concentric square. This was repeated 10 times to obtain one observation per concentric square across the whole slide area, totaling 10 observations in all per slide.



Supplementary Figure 2. Residual plots do not reveal deviations from homoscedasticity, and a histogram and Q-Q plot of residuals do not indicate any violation of normality for the generalized linear mixed effects model.

Appendix B

Chapter 3 supplementary information

Supplementary Tables

Supplementary Table 1 Summary of specimen properties and run assignment. [1]

Run 1 | Run 2 | Run 3 | Run 4 | Raw pool only | Cooked pool only

	SPECIMEN	EDIBLE	FIBER	PEEL	STATE	RUN
V. frutescens		%	%	%		
	1	31	24	45	cook	
	2	28	30	43	raw	
	3	31	32	37	cook	
	4	17	16	67	raw	
	5	23	18	59	cook	
	7	22	33	46	cook	
	8	34	14	52	cook	
	9	22	12	66	raw	
	10	19	23	57	raw	
	11	21	21	59	cook	
	12	43	31	26	raw	
E. entennulifa						
	1	50	8	42	cook	
	2	43	14	43	cook	
	3	45	16	40	cook	
	4	37	7	56	cook	
	5	51	13	36	raw	
	6	58	1	41	cook	
	7	65	1	34	raw	
	8	66	0	34	cook	
	9	32	5	64	raw	
	10	47	13	40	raw	
I. transvaalensis [2]						
	1	80	0	20	raw	
	2	76	0	24	raw	
	3	76	0	24	raw	

	4	75	0	25	raw	
	5	77	0	23	cook	
	6	76	0	24	raw	
	7	76	0	24	cook	
	8	74	0	26	cook	
	9	65	0	35	raw	
	10	76	0	24	raw	
	11	72	0	28	raw	
	12	86	0	14	cook	
	13	69	0	31	cook	
	14	66	0	34	raw	
	15	62	0	38	raw	
	16	75	0	25	cook	
	17	66	0	34	raw	
	18	80	0	20	cook	
	19	74	0	26	cook	
	20	70	0	30	raw	
	22	80	0	20	raw	
	23	74	0	26	cook	
	24	71	0	29	raw	
	25	78	0	22	cook	
	26	66	0	34	raw	
	27	70	0	30	raw	
	28	62	0	38	raw	
	29	80	0	20	cook	
	30	53	0	47	raw	
	31	72	0	28	raw	
	32	74	0	26	raw	
V. pseudolablab						
	1	56	23	21	raw	
	2	48	10	42	cook	
	3	46	17	37	raw	
	4	43	11	46	cook	
	5	46	14	40	cook	
	6	54	9	36	cook	
	7	69	11	20	raw	
	8	55	7	38	cook	
	9	61	16	23	raw	
	10	52	0	48	cook	
	11	63	14	23	raw	
	12	30	31	40	raw	
	13	58	18	24	raw	
	14	45	14	41	cook	

[1] There were six total runs per species: 2 raw, 2 cooked, 1 pooled raw, and 1 one pooled cooked. For our purposes, run numbers are effective sample numbers (n), and are referred to in lieu of specimen number in the rest of the paper for simplicity.

[2] All *I. transvaalensis* runs constitute homogenization of all tubers except for runs 3 and 4 (delineated).

Supplementary Table 2 Comparison summary of Hadza tuber nutritional values across three studies: this study (**a**), Schoeninger et al., 2001 (**b**), and Crittenden, 2009 (**c**).

a.

this study			**g kg^{-1} edible wet weight**	
specimen	**taxonomy**	**state**	**glucose**	**protein**
//ekwa 1	*V. frutescens*	Cook	11.01	21.71
//ekwa 2	*V. frutescens*	Cook	29.67	22.93
//ekwa 3	*V. frutescens*	Raw	27.36	9.42
//ekwa 4	*V. frutescens*	Raw	51.70	31.81
//ekwa 5	*V. frutescens*	Cook	17.42	45.49
//ekwa 6	*V. frutescens*	Raw	19.31	22.42
mak'alitako 1	*E. entennulifa*	Cook	46.40	29.57
mak'alitako 2	*E. entennulifa*	Cook	5.51	1.69
mak'alitako 3	*E. entennulifa*	Raw	6.14	7.67
mak'alitako 4	*E. entennulifa*	Raw	2.86	3.20
mak'alitako 5	*E. entennulifa*	Cook	9.06	18.01
mak'alitako 6	*E. entennulifa*	Raw	5.75	11.67
panjuko 1	*I. transvaalensis*	Cook	61.25	6.66
panjuko 2	*I. transvaalensis*	Cook	114.86	33.00
panjuko 3	*I. transvaalensis*	Raw	86.35	18.29
panjuko 4	*I. transvaalensis*	Raw	140.46	14.51
panjuko 5	*I. transvaalensis*	Cook	45.23	27.52
panjuko 6	*I. transvaalensis*	Raw	22.52	21.54
shumuko 1	*V. pseudolablab*	Cook	17.10	17.48
shumuko 2	*V. pseudolablab*	Cook	14.68	9.68
shumuko 3	*V. pseudolablab*	Raw	12.46	8.34
shumuko 4	*V. pseudolablab*	Raw	18.99	12.71
shumuko 5	*V. pseudolablab*	Cook	6.47	35.13
shumuko 6	*V. pseudolablab*	Raw	14.13	42.78

b.

Schoeninger et al., 2001			g 100g^{-1} edible dry weight		g kg^{-1} edible wet weight	
specimen	**taxonomy**	**% moisture**	**carbohy-drate**	**protein**	**carbohy-drate**	**protein**
//ekwa (avg)	*V. frutescens*	78	32.20	4.50	71.81	10.04
mak'alitako	*E. entennulifa*	80	42.80	6.90	86.03	13.87
panjuko	*I. transvaalensis*	86	72.20	2.30	101.80	3.24
//ekwa 1	*V. frutescens*	78	61.80	4.20	137.81	9.37
//ekwa 2	*V. frutescens*	83	32.00	4.40	53.12	7.30
//ekwa 3	*V. frutescens*	75	37.80	3.30	94.12	8.22
//ekwa 4	*V. frutescens*	77	20.10	4.20	47.24	9.87
//ekwa 5a	*V. frutescens*	76	8.20	5.60	19.84	13.55
//ekwa 5b	*V. frutescens*	76	9.90	6.80	23.96	16.46

c.

Crittenden, 2009				g 100g^{-1} peeled dry weight		g kg^{-1} peeled wet weight	
specimen	**taxonomy**	**state**	**% moisture**	**carbohy-drate**	**protein**	**carbohy-drate**	**protein**
//ekwa	*V. frutescens*	Raw	69	75.40	7.90	231.48	24.25
//ekwa	*V. frutescens*	Raw	68	88.80	4.80	285.94	15.46
//ekwa	*V. frutescens*	Raw	69	80.80	9.00	247.25	27.54
//ekwa	*V. frutescens*	Raw	70	63.20	7.10	190.86	21.44
//ekwa	*V. frutescens*	Cook	68	68.30	7.60	220.61	24.55
//ekwa	*V. frutescens*	Cook	69	82.80	10.40	259.16	32.55
//ekwa	*V. frutescens*	Cook	69	60.50	5.90	185.74	18.11
//ekwa	*V. frutescens*	Cook	69	94.70	5.10	297.36	16.01
shumuko	*V. pseudolablab*	Raw	89	62.90	3.00	71.08	3.39
shumuko	*V. pseudolablab*	Cook	91	63.10	3.60	54.27	3.10
makalita	*R. comosa*	Raw	79	60.20	5.40	124.61	11.18
makalita	*R. comosa*	Raw	79	37.40	5.90	78.17	12.33
makalita	*R. comosa*	Cook	79	54.30	5.10	112.40	10.56
matukwaya	*C. surantiaca*	Raw	87	92.60	12.40	125.01	16.74
shaehako	*V. macrorhyncha*	Raw	86	69.00	10.40	98.67	14.87

MICRONUTRIENT ASSAY METHODS

According to ISO-(N)EN-AOAC method

Analysis method

SOP-code

Technique

Description

Vitamin B1

TRIS/VIT/010

HPLC-Fluorescence

Samples are extracted in duplicate in an autoclave at 119°C for 15 minutes, using a 0.1 mol/l hydrochloric acid solution containing sodium ascorbate. The resulting extract is adjusted to pH 4.7 using an acetate buffer, and filtrated. Part of the filtrate is incubated for at least 2 hours at 37°C with phosphatase(acid), and subsequently analyzed for thiamin using reversed phase UPLC with post-column derivatisation and fluorescence detection.

Vitamin B2

TRIS/VIT/012

HPLC-Fluorescence

Samples are extracted in duplicate in an autoclave at 119°C for 15 minutes, using a 0.1 mol/l hydrochloric acid solution containing sodium ascorbate. The resulting extract is adjusted to pH 4.7 using an acetate buffer, and filtrated. Part of the filtrate is incubated for at least 2 hours at 37°C with phosphatase (acid), and subsequently analyzed for riboflavin using reversed phase UPLC with fluorescence detection.

Vitamin B3

TRIS/VIT/209

LC-MS/MS

Samples are extracted in duplicate in an autoclave at 119°C for 15 minutes, using a 0.1 mol/l hydrochloric

acid solution containing sodium ascorbate. The resulting extract is adjusted to pH 4.7 using an acetate buffer, and filtrated. Part of the filtrate is subsequently analyzed for nicotinamide and nicotinic acid using reversed phase UPLC with MS/MS detection.

Pantothenic Acid

TRIS/VIT/035

LC-MS/MS

Samples are analyzed in duplicate. First, samples are dissolved in pantothenic acid and enzymatically liberated in an extraction buffer containing papain and taka-diastase. Subsequently, the extraction process is continued in an autoclave at 119°C for 15 minutes. The resulting extract is filtrated and further diluted for analysis by reversed phase chromatography with tandem mass-spectrometry. Isotopically labeled pantothenic acid is used as internal standard.

Vitamin B6

TRIS/VIT/015

HPLC-Fluorescence

Samples are extracted in duplicate for 30 minutes, using a 5% trichloroacetic acid solution. A part of the extract is adjusted to pH 4.7 using an acetate buffer, and incubated for at least 12 hours at 37°C with phosphatase (acid), and subsequently analyzed for pyridoxal, pyridoxol and pyridoxamin using reversed phase UPLC with fluorescence detection.

Biotin

TRIS/VIT/206

LC-MS/MS

Samples are analyzed in duplicate in a series with a Quality Control sample (Infant Nutrition). Biotin is extracted using sulphuric acid (pH 4.5) at 119°C during 30 minutes. After filtration and dilution, biotin is determined using UPLC-MS/MS with isotopically labeled biotin (ring 6,6 d2) as internal standard.

Total Folate

TRIS/VIT/107

Microbiology

A liquid sample extract is added to a nutrient medium. This nutrient medium is free of the compound of interest but contains all the ingredients for the bacteria to grow. After grow of the bacteria in the sample extract grow is compared with grow of bacteria in a standard solution with a known concentration.

Vitamin B12

TRIS/VIT/065

RIA

Samples are extracted in duplicate in an autoclave at 119°C for 15 minutes, using an 0.1 M acetate buffer pH 4.6 containing 50 mg/l potassium cyanide. This procedure converts all cobalamines to the cyanocobalamine vitamer. The filtrated extract is diluted to appropriate concentrations for the competitive binding assay, and incubated for 1 hour with 57Co-cyanocobalamine, intrinsic factor, and a glutaminic acid buffer pH 4.1. To test for parallelism, four serial dilutions are being used for each of the duplicates. Subsequently, a cold activated carbon solution is added, and after vigorous mixing the solution is left for 10 minutes. The slurry is centrifuged, and the supernatant analyzed using a Wallac Wizard 1470 gamma-counter.

Vitamin C

TRIS/VIT/014

HPLC-Fluorescence

Vitamin C is extracted from the sample using trichloro acetic acid. Ascorbic acid is oxidized into dehydro-ascorbic acid which is subsequently condensed into its quinoxaline derivative using 1,2-diamino benzene. This derivative is measured using UPLC with fluorescence detection.

Vitamin A

TRIS/VIT/113

HPLC-Fluorescence

Samples are saponified in duplicate under reflux conditions for 30 minutes, using a 1.5 mol/l potassium hydroxide ethanolic solution supplemented with sodium ascorbate and disodium sulfide. After cooling at room

temperature, the mixture is extracted using diisopropyl ether, which is subsequently washed with aqueous solutions. The resulting extract is analyzed for all-trans retinol and 13-cis retinol using straight phase HPLC with fluorescence detection.

Vitamin D

TRIS/VIT/051

HPLC-DAD

Samples, to which vitamin D2 is added as internal standard, are saponified in duplicate under reflux conditions for 30 minutes, using a 1.5 mol/l potassium hydroxide ethanolic solution supplemented with sodium ascorbate and disodium sulfide. After cooling at room temperature, the mixture is extracted using diisopropyl ether, which is subsequently washed with aqueous solutions. The resulting extract is partly evaporated and redissolved in methanol. Subsequently, the vitamin D in the eluate is further purified by fractionation with straight phase chromatography. Finally, the resulting extract is analyzed by reversed phase chromatography with diode array detection, quantifying at 265 nm.

Vitamin E

TRIS/VIT/113

HPLC-Fluorescence

Samples are saponified in duplicate under reflux conditions for 30 minutes, using a 1.5 mol/l potassium hydroxide ethanolic solution supplemented with sodium ascorbate and disodium sulfide. After cooling at room temperature, the mixture is extracted using diisopropyl ether, which is subsequently washed with aqueous solutions. The resulting extract is analyzed for α-tocopherol using straight phase HPLC with fluorescence detection.

Vitamin K1

TRIS/VIT/021

HPLC-Fluorescence

Samples are dissolved in duplicate as aqueous solution, after which they are enzymatically digested using pancreatin. Subsequently, vitamin K1 is extracted using n-pentane. The extract is purified using a normal

phase SPE procedure, after which vitamin K1 is analyzed using reversed phase HPLC with post-column Zn-reduction and fluorescence detection.

Carotenoids

TRIS/VIT/058

HPLC-DAD

Samples are saponified in duplicate under reflux conditions for 30 minutes, using a 1.5 mol/l potassium hydroxide ethanolic solution supplemented with sodium ascorbate and disodium sulfide. After cooling at room temperature, the mixture is extracted using diisopropyl ether, which is subsequently washed with aqueous solutions. The resulting extract is analyzed for beta-carotene using straight phase HPLC with diode array detection.

Minerals / Trace Elements

TRIS/LSP/055

ICP-MS

For the determination of the minerals calcium, sodium, phosphorus, magnesium, sulfur and potassium and the elements iron, zinc, copper, manganese, cobalt, chromium, molybdenum and selenium, a test portion of the sample is digested with nitric acid using a digestion block. The concentration of the elements is determined by inductively coupled plasma mass spectrometry (ICP-MS) using external calibration.

Ash

TRIS/LNC/167

Incineration at 550 °C Samples are dry ashed and the residue is weighed.

Chloride and Iodide

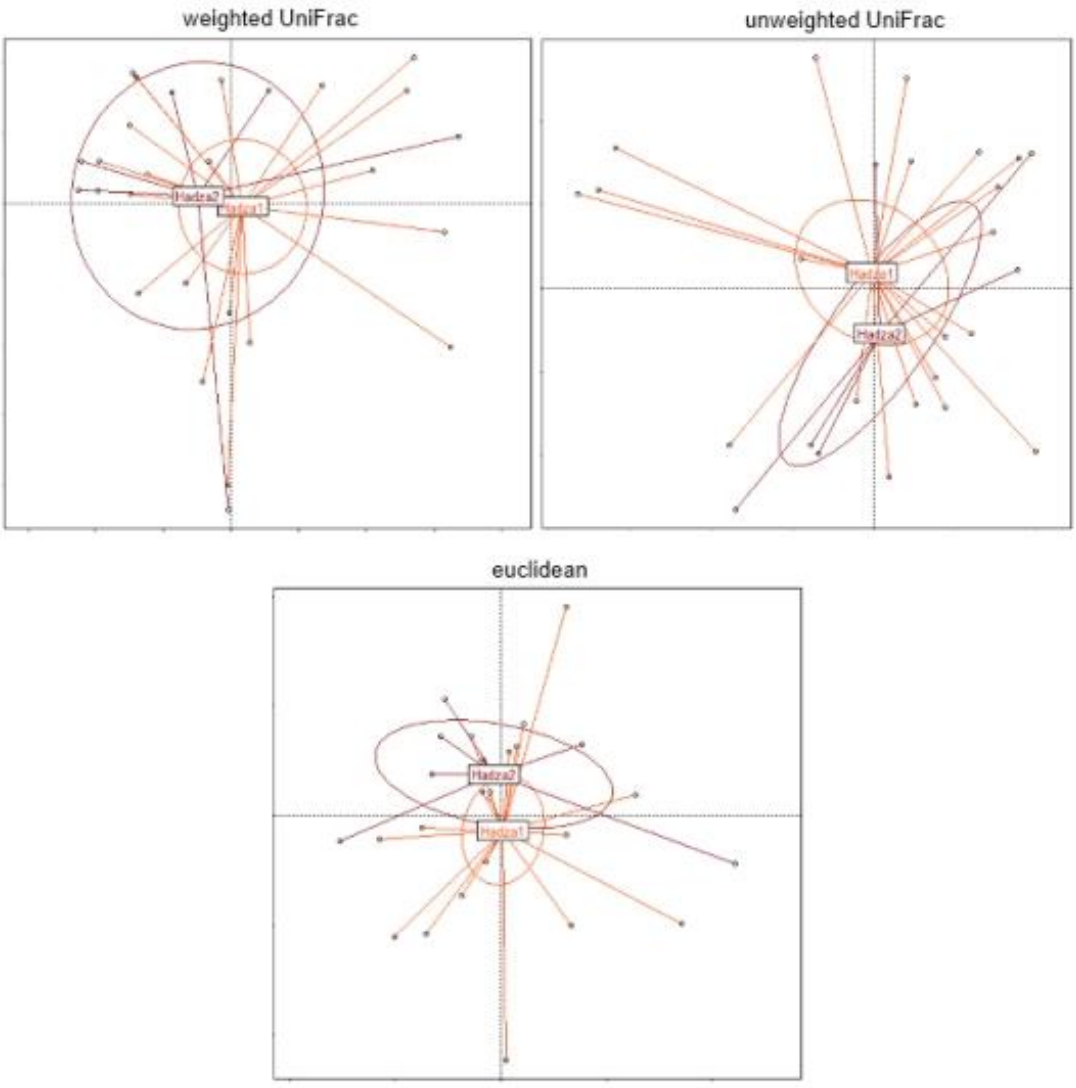
TRIS/LSP/055

ICP-MSFor the determination of Iodine and Chloride, a test portion of the sample is extracted with diluted ammonia. The concentration of Iodine and Chloride is determined by inductively coupled plasma atomic mass spectrometry (ICP-MS) using an internal standard. Quantification is performed with external calibration.

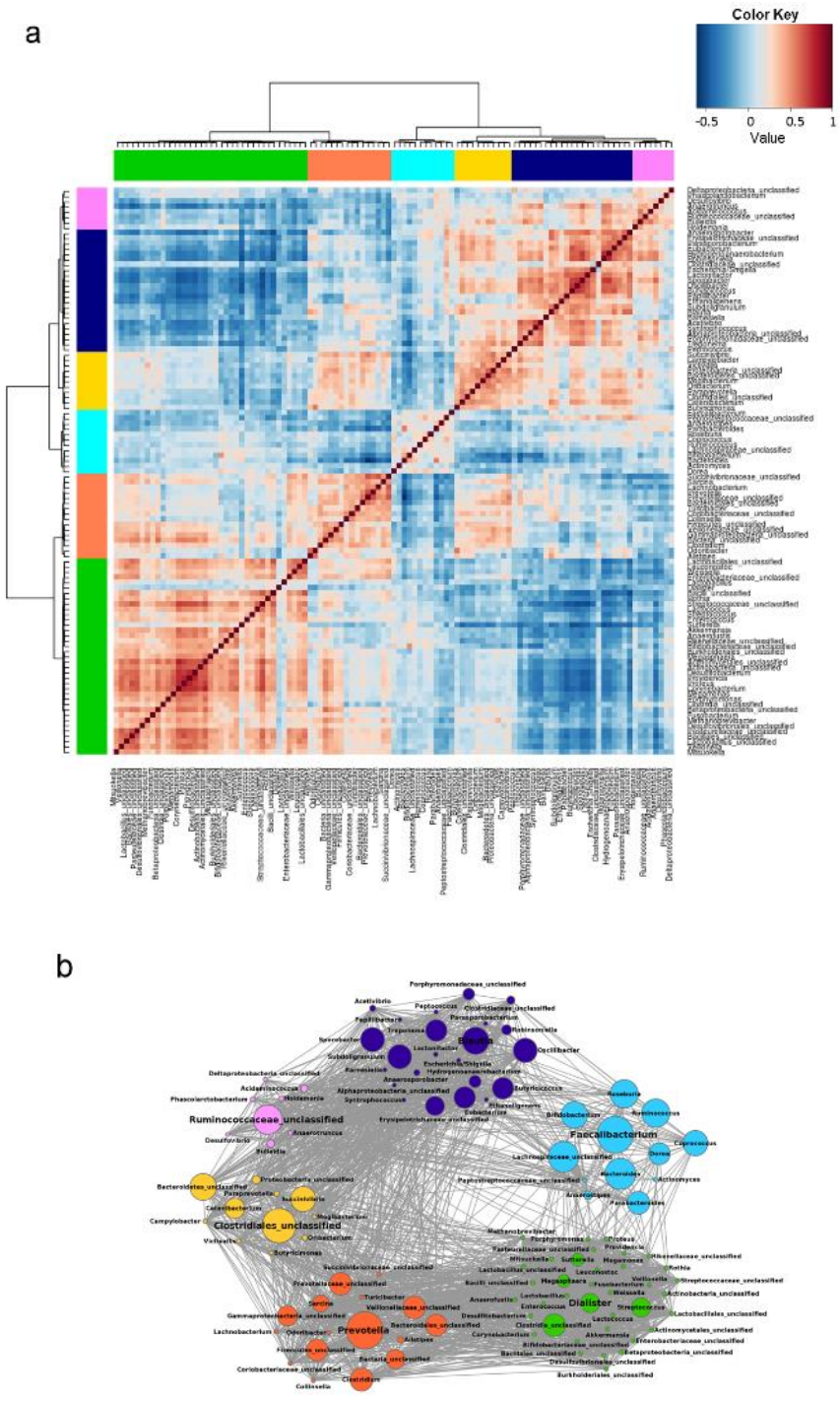
Appendix C

Chapter 4 supplementary information

Supplementary Figures



Supplementary Figure 1. Phylogenetic diversity within the Hadza gut microbial community. PCoA plots based on weighted and unweighted UniFrac distances and Euclidean distances of the genus level relative abundances are shown. Subjects are grouped according to camp provenience, Dedauko (Hadza 1) and Sengele (Hadza 2). None of the diversity metrics show significant separation of the Hadza gut microbiota by camp location.



Supplementary Figure 2. Assignments of bacterial co-abundance groups (CAGs). CAG assignment relied on a heat plot (**a**) showing Kendall correlations between genera clustered by the Spearman correlation coefficient and Ward linkage hierarchical clustering. Colors are indicative of the six identified CAGs. (**b**) Wiggum plot correlations between the six CAGs identified, circle size is representative of the genus abundance and the connections between nodes represent positive and significant Kendall correlations between genera (FDR < 0.05).

Supplementary Tables

Supplementary Table 1. qPCR quantification of *Bifidobacterium* in stool samples. For each subject, the *Bifidobacterium* 16S rRNA copy number μg^{-1} of fecal DNA is reported. BDL: Below detection limit (100 16S rRNA gene copies ng^{-1} faecal DNA)

Hadza		**Italians**	
Subject	16S rRNA gene copies ng^{-1} faecal DNA	Subject	16S rRNA gene copies ng^{-1} faecal DNA
H1	BDL	IT1	6.55E+05
H2	BDL	IT2	1.41E+06
H3	BDL	IT3	1.39E+06
H4	BDL	IT4	1.38E+04
H5	BDL	IT5	1.19E+06
H6	BDL	IT6	2.05E+05
H7	BDL	IT7	1.22E+05
H8	BDL	IT8	1.88E+05
H9	BDL	IT9	1.50E+05
H10	BDL	IT10	6.55E+04
H11	BDL	IT11	7.53E+04
H12	BDL	IT12	5.48E+05
H13	BDL	IT13	2.36E+05
H14	BDL	IT14	1.56E+05
H15	BDL	IT15	1.82E+05
H16	BDL	IT16	1.30E+05
H17	BDL		
H18	BDL		
H19	BDL		
H20	BDL		
H21	BDL		
H22	BDL		
H23	BDL		
H24	BDL		
H25	BDL		
H26	BDL		
H27	BDL		
H28	BDL		

Supplementary Table 2. Absolute and percentage based values of SCFAs in Hadza and Italian samples.

a.

HADZA	Acetic	Propionic	Butyric	Valeric
	(umol g^{-1})	(umol g^{-1})	(umol g^{-1})	(umol g^{-1})
H16	1.4	0.27	0.12	0.025
H1	2.3	0.71	0.33	0.063
H14	2.7	1.06	0.79	0.104
H2	2.3	0.67	0.69	0.119
H13	4.7	1.71	1.26	0.126
H11	4.9	1.81	1.19	0.141
H12	4.3	1.90	1.65	0.181
H10	4.8	1.38	0.71	0.108
H15	2.4	0.98	0.77	0.114
H6	3.5	1.46	0.80	0.070
H5	5.2	0.96	0.32	0.091
H4	1.8	0.52	0.33	0.096
H8	4.5	1.40	0.57	0.075
H7	4.0	1.55	0.70	0.098
H3	4.8	1.01	0.67	0.129
H9	4.3	0.98	0.44	0.063
H22	1.9	0.39	0.27	0.043
H19	2.2	0.50	0.41	0.054
H17	4.5	2.00	1.79	0.177
H21	3.4	0.71	0.48	0.063
H20	3.4	1.09	0.23	0.061
H18	2.2	0.56	0.36	0.058
H23	1.5	0.40	0.34	0.040
H25	2.2	0.87	0.67	0.082
H24	3.9	1.85	0.63	0.075
H26	0.8	0.17	0.13	0.027
H28	0.8	0.17	0.06	0.021
H27	0.8	0.17	0.11	0.021

b.

ITALIANS	Acetic	Propionic	Butyric	Valeric
	(umol g^{-1})	(umol g^{-1})	(umol g^{-1})	(umol g^{-1})
IT11	1.1	0.43	0.20	not determined
IT1	9.5	2.20	2.64	0.168
IT6	2.2	0.41	0.65	0.045
IT3	3.1	0.75	1.52	0.075
IT5	1.7	0.39	0.30	0.083
IT2	1.1	0.10	0.13	0.039
IT4	0.7	0.17	0.18	0.030
IT12	1.7	0.25	0.46	0.021
IT8	1.4	0.40	0.36	0.038
IT14	0.4	0.06	0.05	0.010
IT10	1.3	0.34	0.35	0.048
IT7	3.5	0.71	1.20	0.056
IT16	1.8	0.58	0.20	0.003
IT15	2.9	0.68	0.81	0.076
IT9	0.7	0.17	0.22	0.024
IT13	5.2	0.91	1.53	0.068

HADZA	TOTAL (µmol g^{-1})	Acetic	Propionic	Butyric	Valeric
		%	%	%	%
H16	1.83	77.23	14.56	6.82	1.39
1H	3.42	67.89	20.76	9.53	1.83
H14	4.69	58.34	22.55	16.90	2.21
H2	3.77	60.71	17.80	18.34	3.15
H13	7.80	60.23	21.96	16.19	1.62
H11	8.04	60.95	22.51	14.78	1.76
H12	8.07	53.86	23.51	20.39	2.24
H10	6.96	68.38	19.80	10.25	1.56
H15	4.31	56.67	22.81	17.89	2.64
H6	5.84	60.17	24.92	13.72	1.19
H5	6.53	79.01	14.74	4.85	1.40
H4	2.74	65.63	18.91	11.97	3.50
H8	6.57	68.95	21.24	8.67	1.14
H7	6.34	62.93	24.43	11.09	1.55
H3	6.60	72.57	15.37	10.10	1.96
H9	5.76	74.32	17.03	7.56	1.09
H22	2.63	73.07	15.01	10.27	1.65
H19	3.15	69.40	15.96	12.92	1.71
H17	8.42	52.87	23.75	21.27	2.11
H21	4.66	73.02	15.27	10.36	1.35
H20	4.81	71.35	22.55	4.83	1.27
H18	3.18	69.06	17.73	11.38	1.83
H23	2.24	65.24	17.80	15.20	1.76
H25	3.85	57.66	22.70	17.52	2.13
H24	6.43	60.27	28.81	9.76	1.16
H26	1.12	70.57	15.22	11.79	2.42
H28	1.07	76.45	16.08	5.45	2.01
H27	1.06	71.64	15.62	10.75	1.99

ITALIANS	TOTAL (µmol g^{-1})	Acetic	Propionic	Butyric	Valeric
		%	%	%	%
IT11	1.75	63.59	24.79	11.62	not determined
IT1	14.55	65.55	15.13	18.16	1.16
IT6	3.27	66.11	12.68	19.85	1.36
IT3	5.43	56.81	13.89	27.93	1.37
IT5	2.50	69.04	15.49	12.15	3.33
IT2	1.35	79.97	7.52	9.62	2.89
IT4	1.09	65.64	15.26	16.36	2.74
IT12	2.46	70.62	10.00	18.53	0.85
IT8	2.23	63.95	18.10	16.24	1.70
IT14	0.49	73.59	13.20	11.13	2.07
IT10	2.08	64.58	16.45	16.66	2.31
IT7	5.52	64.22	12.90	21.86	1.03
IT16	2.58	69.77	22.38	7.72	0.13
IT15	4.49	65.13	15.18	18.00	1.68
IT9	1.09	62.53	15.34	19.94	2.19
IT13	7.72	67.44	11.84	19.84	0.88

Supplementary Table 3. Correlations between SCFA levels and genera relative abundance. Correlations were carried out using Kendall correlation tests in R. Bacterial groups are highlighted and marked by double asterisk (**) if they are correlated, positively or negatively, with SCFA level and if they are present at 5% or more of total abundance in at least one of the two populations. Results reported in the table show that SCFAs are correspondingly enriched and depleted with certain bacterial groups that are differently represented in the Hadza and Italian GM. Propionate levels associated most strongly with bacterial genera that are enriched in Hadza and depleted in Italians whereas butyrate levels were positively associated with bacteria enriched in Italians and depleted in Hadza. These correlations inform us of the functional traits established in each community GM and support the finding that the Hadza GM community is structured around production of propionate whereas the Italian GM is better suited to butyrate production.

Acetate

taxa	tau	P	ITA	±SEM	HADZA	±SEM	P value (Kendall tau rank correlation coefficient)
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae;Paludibacter	-0.2699	3.55E-02	0	0	0.02	0.01	7.00E-03
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Chitinophagaceae;Flavisolibacter	0.2781	3.96E-02	0	0	0	0	2.90E-01
Bacteria;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Sarcina	0.3957	1.79E-03	0	0	0.08	0.04	3.40E-02
Bacteria;Firmicutes;Clostridia;Clostridiales;Incertae Sedis XI;Anaerococcus	0.3038	2.28E-02	0.01	0.01	0	0	6.70E-01
Bacteria;Firmicutes;Clostridia;Clostridiales;Incertae Sedis XII;Acidaminobacter	0.2986	1.57E-02	0.03	0.02	0.37	0.22	1.60E-02
Bacteria;Firmicutes;Clostridia;Clostridiales;Incertae Sedis XIII;Anaerovorax	0.3121	2.01E-02	0	0	0	0	9.10E-01
Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;Robinsoniella	0.2773	1.54E-02	0.02	0	0.86	0.2	1.30E-06
Bacteria;Firmicutes;Clostridia;Clostridiales;Ruminococcaceae;Faecalibacterium**	-0.4367	7.24E-05	18.46	2.4	11.79	1.03	1.10E-02
Bacteria;Firmicutes;Clostridia;Clostridiales;Veillonellaceae;Megasphaera	0.2836	3.02E-02	0.23	0.2	0.02	0.01	8.40E-01
Bacteria;Lentisphaerae;Lentisphaeria;Victivallales;Victivallaceae;Victivallis	0.2951	1.76E-02	0.01	0.01	0.02	0.01	7.20E-03
Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;Buttiauxella	-0.3482	9.06E-03	0	0	0	0	6.00E-01
Bacteria;Proteobacteria;Gammaproteobacteria;Pasteurellales;Pasteurellaceae;Mannheimia	-0.2949	2.33E-02	0	0	0.01	0.01	6.00E-01

Valerate

taxa	tau	P	ITA	±SEM	HADZA	±SEM	P value (Kendall tau rank correlation coefficient)
Bacteria;Firmicutes;Bacilli;Lactobacillales;Lactobacillaceae;Lactobacillus	-0.2708	2.84E-02	0.02	0.01	0.06	0.04	5.10E-01
Bacteria;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Oxobacter	0.2882	1.97E-02	0.06	0.02	0.02	0.01	6.80E-02
Bacteria;Firmicutes;Clostridia;Clostridiales;Incertae Sedis XI;Sedimentibacter	-0.2781	3.96E-02	0	0	0	0	2.30E-02
Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;Other**	-0.4651	2.12E-05	6.41	0.69	3.86	0.24	5.50E-04
Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;Roseburia**	-0.3741	7.77E-04	7.73	1.3	3.88	0.77	7.00E-03
Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;Syntrophococcus	0.2991	1.32E-02	0	0	0.07	0.02	2.50E-05
Bacteria;Firmicutes;Clostridia;Clostridiales;Other;Other**	0.2632	2.00E-02	7.37	0.79	11.13	0.56	2.20E-04

Bacteria;Firmicutes;Clostridia;Clostridiales;Peptococcaceae;Peptococcus	0.2648	3.01E-02	0.06	0.04	0.04	0.01	1.10E-02
Bacteria;Firmicutes;Clostridia;Clostridiales;Ruminococcaceae;Acetivibrio	0.2765	1.80E-02	0.01	0	0.4	0.07	2.70E-06
Bacteria;Firmicutes;Clostridia;Clostridiales;Ruminococcaceae;Hydrogenoanaerobacterium	0.234	4.10E-02	0.07	0.03	0.97	0.13	9.90E-06
Bacteria;Firmicutes;Clostridia;Clostridiales;Ruminococcaceae;Oscillibacter	0.2688	1.74E-02	1.06	0.28	3.79	0.42	1.00E-05
Bacteria;Firmicutes;Clostridia;Clostridiales;Veillonellaceae;Dialister	-0.3434	4.67E-03	0.09	0.04	0.06	0.02	8.70E-01
Bacteria;Firmicutes;Clostridia;Other;Other;Other	0.279	1.45E-02	0.03	0.02	0.35	0.04	9.90E-07
Bacteria;Other;Other;Other;Other;Other	0.266	1.86E-02	0.22	0.05	1.82	0.16	3.80E-09
Bacteria;Proteobacteria;Deltaproteobacteria;Other;Other;Other	0.3814	2.46E-03	0	0	0.33	0.13	2.70E-02
Bacteria;Proteobacteria;Gammaproteobacteria;Pasteurellales;Pasteurellaceae;Actinobacillus	-0.239	4.52E-02	0.02	0.01	0.11	0.05	1.10E-02

Butyrate

taxa	tau	P	ITA	±SEM	HADZA	±SEM	P value (Kendall tau rank correlation coefficient)
Bacteria;Actinobacteria;Actinobacteria;Bifidobacteriales;Bifidobacteriaceae;Bifidobacterium**	0.2869	1.56E-02	8.13	1.5	0.02	0.01	3.20E-08
Bacteria;Actinobacteria;Actinobacteria;Bifidobacteriales;Bifidobacteriaceae;Other	0.2949	2.33E-02	0.03	0.01	0	0	2.90E-07
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Bacteroidaceae;Bacteroides**	0.3087	6.04E-03	7.14	1.95	0.21	0.04	3.40E-08
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Other;Other	-0.2603	2.14E-02	0.07	0.02	0.79	0.11	1.50E-09
Bacteria;Bacteroidetes;Other;Other;Other;Other**	-0.248	2.87E-02	0.15	0.07	6.57	0.81	8.10E-08
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Chitinophagaceae;Flavisolibacter	-0.2781	3.96E-02	0	0	0	0	2.90E-01
Bacteria;Fibrobacteres;Fibrobacteria;Fibrobacterales;Fibrobacteraceae;Fibrobacter	-0.2758	3.99E-02	0	0	0	0	1.80E-01
Bacteria;Firmicutes;Bacilli;Lactobacillales;Enterococcaceae;Enterococcus	0.2779	3.15E-02	0.03	0.01	0	0	1.00E-04
Bacteria;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Sarcina	-0.4578	3.04E-04	0	0	0.08	0.04	3.40E-02
Bacteria;Firmicutes;Clostridia;Clostridiales;Gracilibacteraceae;Lutispora	-0.2724	3.05E-02	0.01	0.01	0.03	0.01	9.90E-03
Bacteria;Firmicutes;Clostridia;Clostridiales;Incertae Sedis XII;Acidaminobacter	-0.3298	7.62E-03	0.03	0.02	0.37	0.22	1.60E-02
Bacteria;Firmicutes;Clostridia;Clostridiales;Incertae Sedis XIII;Mogibacterium	-0.2813	2.05E-02	0	0	0.04	0.01	2.80E-05
Bacteria;Firmicutes;Clostridia;Clostridiales;Incertae Sedis XIV;Blautia**	0.3201	4.35E-03	9.45	1.25	3.46	0.4	8.60E-08
Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;Dorea	0.303	7.09E-03	1.35	0.2	0.41	0.06	4.60E-07
Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;Marvinbryantia	-0.2869	3.37E-02	0	0	0	0	3.20E-01
Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;Robinsoniella	-0.3408	2.91E-03	0.02	0	0.86	0.2	1.30E-06
Bacteria;Firmicutes;Clostridia;Clostridiales;Ruminococcaceae;Anaerotruncus	-0.2329	4.03E-02	0.07	0.02	0.32	0.04	5.20E-05
Bacteria;Firmicutes;Clostridia;Clostridiales;Ruminococcaceae;Faecalibacterium**	0.5192	1.53E-06	18.46	2.4	11.79	1.03	1.10E-02

Bacteria;Firmicutes;Clostridia;Clostridiales;Ruminococcaceae;Oscillibacter	-0.2688	1.74E-02	1.06	0.28	3.79	0.42	1.00E-05
Bacteria;Firmicutes;Clostridia;Clostridiales;Ruminococcaceae;Ruminococcus**	0.2916	9.66E-03	8.55	1.82	2.05	0.29	2.70E-03
Bacteria;Firmicutes;Clostridia;Clostridiales;Veillonellaceae;Other	-0.2473	3.02E-02	0.02	0.01	0.83	0.17	3.00E-07
Bacteria;Firmicutes;Erysipelotrichi;Erysipelotrichales;Erysipelotrichaceae;Other	0.3428	2.18E-03	1.49	0.33	0.7	0.16	1.30E-02
Bacteria;Lentisphaerae;Lentisphaeria;Victivallales;Victivallaceae;Victivallis	-0.3378	6.60E-03	0.01	0.01	0.02	0.01	7.20E-03
Bacteria;Proteobacteria;Alphaproteobacteria;Other;Other;Other	-0.2311	4.69E-02	0.02	0.01	0.25	0.07	1.20E-04
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Methylobacteriaceae;Other	-0.2958	2.86E-02	0	0	0	0	2.90E-01
Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Alcaligenaceae;Sutterella	0.3051	2.14E-02	0.03	0.02	0	0	7.90E-04
Bacteria;Proteobacteria;Epsilonproteobacteria;Campylobacterales;Campylobacteraceae;Campylo-bacter	-0.3054	1.01E-02	0	0	0.24	0.07	1.20E-06
Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;Buttiauxella	0.2849	3.27E-02	0	0	0	0	6.00E-01
Bacteria;Proteobacteria;Other;Other;Other;Other	-0.2917	1.08E-02	0.02	0.01	0.52	0.15	1.60E-07
Bacteria;Spirochaetes;Spirochaetes;Spirochaetales;Spirochaetaceae;Treponema	-0.2831	1.37E-02	0	0	2.5	0.72	4.40E-08

Propionate

taxa	tau	P	ITA	±SEM	HADZA	±SEM	P value (Kendall tau rank correla-tion coefficient)
Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Actinomycetaceae;Actinomyces	-0.2734	2.35E-02	0.02	0.01	0.01	0	2.20E-01
Bacteria;Actinobacteria;Actinobacteria;Bifidobacteriales;Bifidobacteriaceae;Bifidobacterium**	-0.3609	2.35E-03	8.13	1.5	0.02	0.01	3.20E-08
Bacteria;Actinobacteria;Actinobacteria;Bifidobacteriales;Bifidobacteriaceae;Other	-0.4469	5.87E-04	0.03	0.01	0	0	2.90E-07
Bacteria;Actinobacteria;Actinobacteria;Coriobacteriales;Coriobacteriaceae;Asaccharobacter	-0.3224	1.44E-02	0.01	0	0	0	4.40E-04
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Marinilabiaceae;Anaerophaga	0.2323	4.48E-02	0	0	0.61	0.1	4.20E-08
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae;Other	0.3062	7.48E-03	0.03	0.02	0.91	0.28	1.50E-06
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae;Paludibacter	0.4205	1.05E-03	0	0	0.02	0.01	7.00E-03
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Prevotellaceae;Prevotella**	0.41	3.05E-04	0.38	0.26	6.23	1.11	5.20E-07
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Prevotellaceae;Xylanibacter	0.288	2.98E-02	0	0	0	0	7.50E-02
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;Alistipes	-0.3626	3.99E-03	0.92	0.22	0	0	2.30E-08
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Rikenellaceae;Other	-0.2758	3.99E-02	0.03	0.02	0	0	7.60E-03
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Other;Other	0.2942	1.22E-02	0	0	0.6	0.11	7.10E-07
Bacteria;Firmicutes;Bacilli;Lactobacillales;Enterococcaceae;Enterococcus	-0.5012	1.05E-04	0.03	0.01	0	0	1.00E-04
Bacteria;Firmicutes;Bacilli;Lactobacillales;Streptococcaceae;Lactococcus	0.2758	3.99E-02	0	0	0	0	1.80E-01
Bacteria;Firmicutes;Clostridia;Clostridiales;Incertae Sedis XI;Finegoldia	-0.3339	1.29E-02	0	0	0	0	2.30E-02

Bacteria;Firmicutes;Clostridia;Clostridiales;Incertae Sedis XIV;Blautia**	-0.2745	1.51E-02	9.45	1.25	3.46	0.4	8.60E-08
Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;Oribacterium	0.3911	7.31E-04	0.01	0	0.31	0.03	7.20E-07
Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;Other**	-0.2432	3.19E-02	6.41	0.69	3.86	0.24	5.50E-04
Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;Pseudobutyrivibrio	0.3147	9.95E-03	0	0	0.04	0.01	1.10E-03
Bacteria;Firmicutes;Clostridia;Clostridiales;Peptostreptococcaceae;Other	-0.2395	3.71E-02	0.35	0.1	0.12	0.03	1.20E-02
Bacteria;Firmicutes;Clostridia;Clostridiales;Ruminococcaceae;Acetanaerobacterium	-0.4224	1.08E-03	0.02	0.01	0	0	1.30E-04
Bacteria;Firmicutes;Clostridia;Clostridiales;Ruminococcaceae;Anaerofilum	-0.352	7.14E-03	0.02	0	0	0	1.90E-05
Bacteria;Firmicutes;Clostridia;Clostridiales;Ruminococcaceae;Butyricicoccus	0.303	7.09E-03	0.37	0.05	2.62	0.47	1.10E-07
Bacteria;Firmicutes;Clostridia;Clostridiales;Veillonellaceae;Other	0.4573	6.15E-05	0.02	0.01	0.83	0.17	3.00E-07
Bacteria;Firmicutes;Erysipelotrichi;Erysipelotrichales;Erysipelotrichaceae;Other	-0.2973	8.29E-03	1.49	0.33	0.7	0.16	1.30E-02
Bacteria;Firmicutes;Erysipelotrichi;Erysipelotrichales;Erysipelotrichaceae;Solobacterium	-0.2672	3.98E-02	0.01	0	0.01	0.01	3.00E-03
Bacteria;Other;Other;Other;Other;Other	0.2262	4.65E-02	0.22	0.05	1.82	0.16	3.80E-09
Bacteria;Proteobacteria;Alphaproteobacteria;Other;Other;Other	0.2756	1.78E-02	0.02	0.01	0.25	0.07	1.20E-04
Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Alcaligenaceae;Parasutterella	-0.4107	1.70E-03	0.05	0.01	0	0	5.00E-06
Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Alcaligenaceae;Sutterella	-0.3336	1.19E-02	0.03	0.02	0	0	7.90E-04
Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Other;Other	0.2779	3.15E-02	0	0	0.01	0.01	5.60E-02
Bacteria;Proteobacteria;Epsilonproteobacteria;Campylobacterales;Campylobacteraceae;Campylo-bacter	0.2745	2.07E-02	0	0	0.24	0.07	1.20E-06
Bacteria;Proteobacteria;Gammaproteobacteria;Aeromonadales;Aeromonadaceae;Aeromonas	0.2781	3.96E-02	0	0	0	0	2.90E-01
Bacteria;Proteobacteria;Gammaproteobacteria;Aeromonadales;Succinivibrionaceae;Ruminobacter	0.3297	9.75E-03	0	0	1.21	0.66	2.90E-02
Bacteria;Proteobacteria;Gammaproteobacteria;Aeromonadales;Succinivibrionaceae;Succinivibrio	0.3499	2.51E-03	0.02	0.02	2.77	0.68	1.10E-07
Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;Other	0.3172	1.60E-02	0	0	0.01	0.01	4.80E-02
Bacteria;Proteobacteria;Other;Other;Other;Other	0.2426	3.40E-02	0.02	0.01	0.52	0.15	1.60E-07
Bacteria;Spirochaetes;Spirochaetes;Spirochaetales;Spirochaetaceae;Treponema	0.3092	7.11E-03	0	0	2.5	0.72	4.40E-08
Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Akkerman-sia	-0.3355	1.19E-02	0.13	0.07	0	0	7.90E-04

Curriculum Vitae

The author of this dissertation was born and raised in North Texas, USA. She received her Bachelor of Arts from Boston University in 2006 in Physical Anthropology, with a Minor in Biology, focusing on neuroscience and endocrinology. In 2008, she enrolled at Texas State University in San Marcos, Texas, to continue graduate education towards her masters in Biological Anthropology, under the advising of Kerrie Lewis Graham, finishing in August 2010. In the Fall of 2011, Stephanie began the PhD program at the Max Planck Institute for Evolutionary Anthropology within the independent research group led by Amanda Henry on Plant Foods in Hominin Dietary Ecology. During the time in Leipzig, Stephanie initiated projects that focused on Hadza women's foraging and consumption of wild tubers in East Africa, building towards future field work in collaboration with and under direction of Alyssa Crittenden at the University of Nevada, Las Vegas. The analysis of Hadza tuber nutrition and gut microbiota were accomplished in collaboration with Koen Venema and Marco Candela respectively, which provided a means of nutritional and molecular laboratory training for Stephanie. Continuing education opportunities in Leipzig were numerous, and included training in starch and phytolith extraction and analysis, collagen extraction for isotope analysis, and programming and statistical computation in R. Off-site training included a Kiswahili language beginner course in Zanzibar, TZ, in-vitro gastro-intestinal digestion and fermentation modeling at TNO in Zeist, NL, beginning bioinformatics community data analysis in Bologna, IT, and procedures for DNA extraction, amplification, quantification, and sequencing at LMAMR in Norman, OK. Throughout the PhD program, Stephanie gave numerous contributed and invited presentations. In 2013, she was awarded the student poster presentation prize at the European Society for the study of Human Evolution (ESHE) in Vienna, and in 2014, the Juan Comas student presentation prize for her podium presentation at the American Association of Physical Anthropologists (AAPA) in Calgary. Publication of the Hadza gut microbiome paper gained considerable attention in the popular press, and Stephanie was invited to contribute a small piece in a double feature, called "Innovations In", on the gut microbiome for the March 2015 issues of *Scientific American* and *Nature*. Peer reviewed publications of the papers that comprise the chapters of this dissertation were published in *Ethnoarchaeology, American Journal of Physical Anthropology,* and *Nature Communications* respectively. Continuing research on the Hadza gut microbiota resulted in a second successful publication on this microbial community, Rampelli et al. 2015 in *Current Biology*, which Stephanie co-authored and completed in tandem with completion of this dissertation work. The journal featured this latter publication on their June 29, 2015 issue cover (see image on the following page). Stephanie currently lives in Norman, OK, USA to pursue postdoctoral research with the Laboratories of Molecular Anthropology and Microbiome Research (LMAMR).

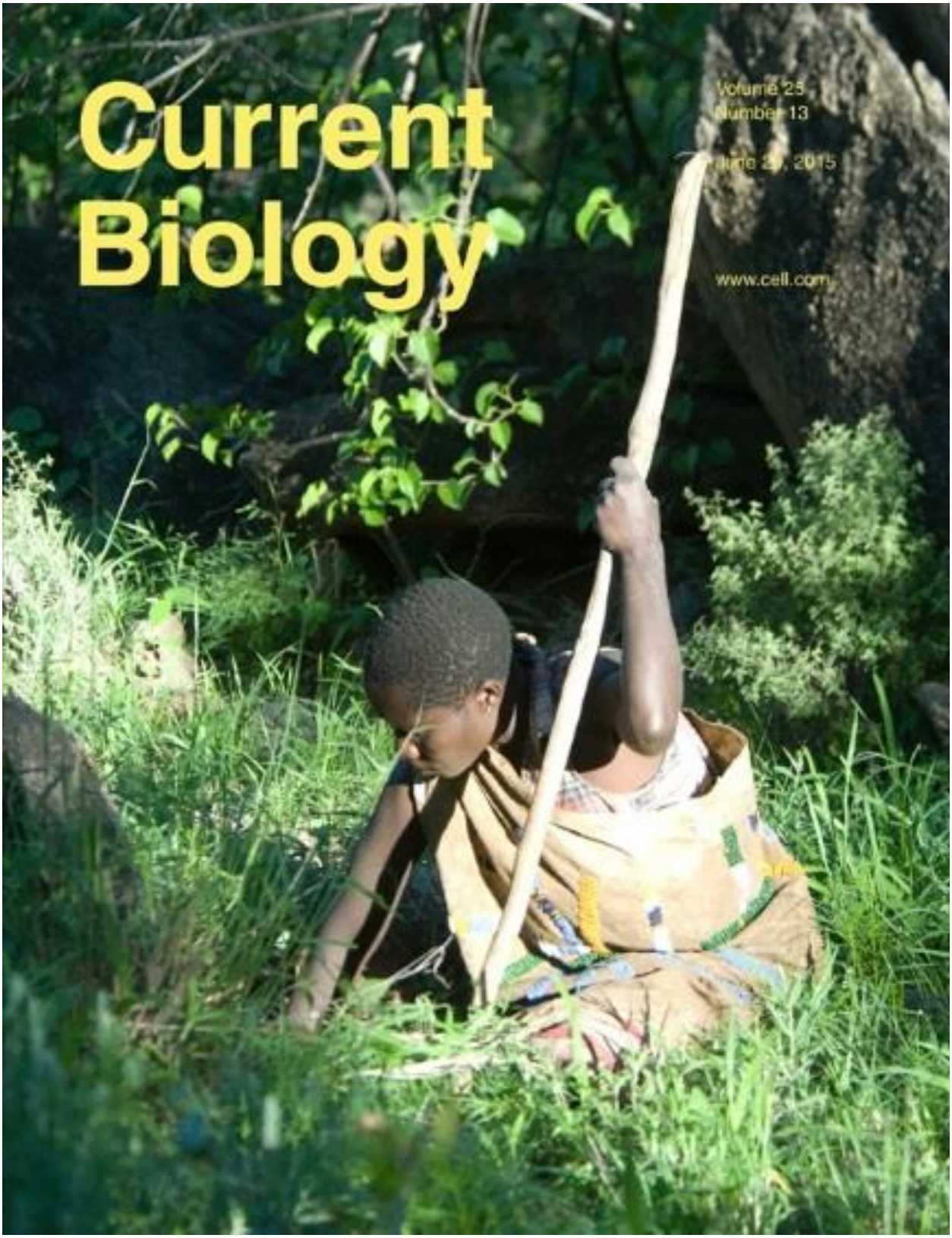


Image 13. Cover feature for June 2015 issue of Current Biology of a Hadza woman digging for wild tubers near Lake Eyasi in Northern Tanzania. Photo taken by Mika Peterson while assisting the author with field research in Tanzania, January 2013.