Big Brain Energy: The Metabolic Contributions of the Gut Microbiome and its Implications for Encephalization in Early *Homo*

by Hannah Jacobson

B.S. in Biological Anthropology, May 2021, The George Washington University

A Thesis submitted to

The Faculty of
The Columbian College of Arts and Sciences
of The George Washington University
in partial fulfillment of the requirements
for the degree of Master of Science

May 15, 2022

Thesis directed by

Brenda Bradley
Associate Professor of Anthropology

Dedication

I would like to dedicate this thesis to my family and those who have been in my corner from the very beginning. To my mother and father, Judy and Eric, who gave nothing but love, support, and enthusiasm, and never questioned me when I said I wanted to study biological anthropology. To my brother, Alex, for his love and his interest and support in all things I have done.

To my godparents, Maggie and Al, for believing in me, cheering me on, and always being around for engaging questions and conversations. I thank them both for their love and their support of my education, both before and during my time at GW.

And, finally, to my grandmother, Joyce, for her love and support, who instilled in me a sense of curiosity and a dedication to learning, and without whom my education and this thesis would not have been possible.

It has been a privilege to share this process with you all, and it is to you all that I owe and dedicate this thesis.

Acknowledgements

I would like to thank my thesis advisor, Dr. Brenda Bradley, for providing support and insights into my work over the past two years and for providing dedicated edits to this final thesis. I would like to thank Dr. Ali Rahnavard for teaching me and for making space in his Public Health lab for an anthropology student who had (previously) limited bioinformatics experience. I am grateful for his edits and insights as my second reader, and I have valued his lessons and knowledge. I would also like to thank the National Science Foundation (Award Number 2109688 and 2028280) and the George Washington University for their financial support of this research.

I would like to give a specific acknowledgement to my friends and colleagues, Katie Auerswald, Nina Lopergolo, and Sarah Grimm. It has been a pleasure learning with and from them, and their company and discourse have made a very challenging year that much easier.

I would also like to acknowledge and thank my friend, Shubha, from whom I have received a great deal of love, support, and insights into public health over the past five years, and whom has received more than her fair share of insights into the gut microbiome in return.

I would like to acknowledge and thank Dr. Shannon McFarlin for all her support and encouragement over my past five years at GW. It is through my introduction to biological anthropology in her lab that I have found my way to where I am now.

It takes a village, and I am lucky enough to have been a part of, and had the support from, an exceptional one at GW and CASHP.

Table of Contents

Dedication.....ii

Acknowledgements.....iii

Table of Contents.....iv

List of Figures……..…..…v

Chapter 1: Introduction……...……1

Chapter 2: The Role of the Microbiome in Digestion and Energy Harvesting.…….....5

Chapter 3: The Evolution of Encephalized *Homo* and the Role of the Gut Microbiome..12

Chapter 4: Background on Data Analysis…..……...18

Chapter 5: Methods…….....21

Chapter 6: Results.....23

Chapter 7: Discussion.....29

Chapter 8: Conclusions.....31

Bibliography.....32

List of Figures

Figure 1: Timeline of early hominin species and the appearance of modern behaviors associated with *Homo* in the fossil record.....14

Figure 2: The Expensive Brain Framework modified to include the metabolic capabilities of the gut microbiome …….....17

Figure 3: Principal Coordinate Analysis of all samples identified by type..... 24

Figure 4: Shannon Index across samples by sample type..…….....25

Figure 5: MaAslin2 output of Lachnospiraceae relative abundance across sample type with significance in the non-industrial group…….....26

Figure 6: MaAslin2 output of Lachnospiraceae relative abundance across sample type with significance in the industrial group…….....…….26

Figure 7: MaAslin2 output of *Ruminococcus champanellensis* relative abundance across sample type with significance in the industrial group.....…..…27

Figure 8: MaAslin2 output of *Ruminococcus champanellensis* relative abundance across sample type with significance in the non-industrial group….....…...27

Figure 9: Heat map of significantly associated features with Paleofecal samples as the control…..…….....…….....……..…28

Chapter 1: Introduction

Our gastrointestinal tract acts as a constant interface between ourselves and the outside world. It is the pathway by which we are in connection with the environment via the food we consume, and it is one of the many direct ways in which the environment can exert evolutionary pressures upon us via the food we eat. The food that we eat is dictated by biome, climate, and culture, and works to both fuel us and shape our biology. One of the notable ways in which our diet dictates who we are is through its influence in determining the structure of our gut microbiome.

Leonard and Robertson (1992) suggested that what an organism eats is a direct reflection of its adaptations to the environment. We, however, are not the sole players in this process of eating, digesting, and metabolizing. Our gut microbiome plays a significant role in the digestion and metabolism of our food, and they themselves are reflective of our environment, diet, and our coevolutionary trajectory over time.

From the moment we ingest food, our bodies work to free as much energy and nutrients as possible from the digestible components. Our gut microbiome plays an important role in this digestion, as well as in the fermentation of the indigestible parts of our diet, and contributes significantly to our overall energy economy. Apart from the extraction of nutrients and the creation of vitamins, the contribution to our energy budget allows our bodies to perform the tasks it needs to survive. The food we eat and the microbes that meet it as it moves into our intestines connect us and our microbiome to the natural.

The human genome and the microbial genome upon which we depend to perform our normal physiological functioning are collectively referred to as the human

metagenome (Turnbaugh and Gordon, 2009). We and our microbes have been coevolving with one another for the past millennia, and we could not survive if our gastrointestinal tract was sterile (O'Hara and Shanahan, 2006).

Our gut microbes contribute to essential functions that our body requires and is unable to produce without them, from metabolic needs to immune function (Blaser, 2006). During our coevolution with these microbes, it is likely they have been selected for their functions in tandem with our nuclear genome. This selection process has resulted in a conserved functional microbiome (Backhed et al., 2012). This is a deep time relationship that cannot be disentangled from our evolutionary history and is likely to have impacted our evolution along the hominin lineage, especially as *Homo* began to manipulate foods through processing with tools, fire, and, eventually, farming.

This thesis will discuss the evolutionary implications of the gut microbiome and its energy harvesting abilities in three distinct but united sections. This first section will review literature on the gut microbiome's metabolic capabilities in order to contextualize their contribution to the host's energy economy through the production of fatty acids. The second section, also a literature review, will discuss the appearance of encephalized *Homo* in the fossil record and the energy frameworks that exist to explain and contextualize this appearance. Both literature reviews establish the context for the third and final section, in which I present the results of an original bioinformatics analysis of previously published data on ancient and modern fecal microbiome data. The goal of this thesis is to suggest changes to the energetic evolutionary frameworks to account for the metabolic capabilities of the gut microbiome and perform a biomarker discovery analysis to identify taxa that may have been selected under this framework.

I posit that the gut microbiome played an overlooked role in energy harvesting that extended the energy budget of early *Homo* to help accommodate a large and energetically expensive brain (Isler and van Schaik, 2009). Microbiome research is being done in a diversity of scientific fields, but, to date, has not been considered for its contribution to the energy budget in frameworks on hominin evolution.

A Note on Terminology

This thesis will utilize coprolite samples collected and given identifying numbers by the authors of the original studies. These numbers are associated with other classifying features, notably industrial and non-industrial samples, which is meant to refer to different populations that eat distinctly different diets. Industrialized, in this context, is meant to refer to populations that eat a diet low in plant-based fibers and high in processed simple carbohydrates and animal products, and non-industrialized is meant to refer to peoples who eat a subsistence-based diet.

I do not feel that the term 'industrialized' is appropriate for these populations, since it generally refers to nations such as the United States and Britain, and other countries that may physically be in the west, but does not take note of nations industrialized through colonialism. It is the western nations such as the United States and Britain that previously colonized other nations and established industrial centers there with the purpose of exploiting natural resources and labor. The nations that were once under colonial rule have industries now that their economies rely on, at least in part. They therefore cannot be considered "non-industrial", since that term would ignore and dismiss their past history under racist and oftentimes violent colonial rule. Industrialized should not be used to refer solely to western and westernized nations

since it was under western nations that many nations (with subsistence-based populations) were forcibly industrialized and exploited.

Here instead I use terms such as westernized to refer to peoples in western or westernized countries and/or who eat a diet that can generally be defined by the following parameters: it is obtained primarily from a supermarket; it is high in animal products raised on an industrial farm; and it is high in simple, processed carbohydrates. The countries included in the original studies as 'industrialized' had diets that satisfied these criteria. For this paper, when I use the term westernize(d), I am referring to the samples originally termed 'industrial' in the original studies. Other diet types, such as agrarian or hunter gatherer, will be discussed as subsistence based strategies. This is an oversimplification of the diversity of diets that are present in either type and in the countries and populations from which the samples originally come, but these broad categories are being generalized to the population level for the purposes of identifying broad trends in the data used for this study.

Chapter 2: The Role of the Microbiome in Digestion and Energy Harvesting

Coevolution and a Core Microbiome

Recent research has shown there is a redundancy in metabolic function between microbes, which accounts for a core functional rather than a core structural microbiome (Lloyd-Price, et al., 2017; Abubucker et al., 2012). A core functional microbiome allows for individual variation in gut structure based on host genetics, environment, food, antibiotic use, and previous colonization (Den Besten et al., 2013). Ultimately, our coevolution with these microorganisms has not required our own genetics to independently evolve these abilities, so we instead rely upon the mutualisms that they confer (Backhed et al., 2005; Ley et al., 2008).

While humans have an overall core functional microbiome (Backhed et al., 2012), the composition of the diet rapidly changes the relative species abundances of the gut flora (David et al., 2014). There is likely a balance between short-term dietary change that influences gut structure and long-term coevolution with our microbes and our diet that influences our core functional microbiome. It will be important to study how major diet shifts within the hominin record have impacted the gut microbiome. It is assumed, for now, that periods of relatively stable dietary intake (over thousands of years of hominin evolution) would influence the structure of our microbiome so that it evolved in tandem to best support the functions of our nuclear genome (Blaser, 2006).

Mutualistic endosymbiont and their functions in the host

This evolved relationship between microbe and host is mutualistic (Backhed et al., 2005), and provides both the host and microbe with fitness benefits (Davenport et al., 2017). The microbes gain a place to live and energy needed to reproduce and the host,

us, gains digestive and metabolic functions, as well as other functions such as immune support.

The gut microbiome plays an essential role in digestion and metabolism and it works in tandem with the host's metabolic processes to contribute to the host's energy balance (Nieuwdorp et al., 2014; Backhed et al., 2007). Without the contributions of the microbiome, we would not be able to digest the more fibrous components of our diet, produce the necessary vitamins our body requires, or extract as much energy from our food as we do (Backhed et al., 2005; Turnbaugh et al. 2006).

Mouse Models as a Foundation

The gut microbiome has been implicated in energy extraction and weight balance through studies on mouse models. Adult mice with no gut microbiome, or germ-free (GF) mice, were underweight and malnourish though they were fed a diet sufficiently high in calories. A fecal transplant from conventionally-raised mice to the GF mice was sufficient to restore the microbiome, at which time the mice were seen to gain weight (Turnbaugh et al., 2006; Backhed et al., 2004). This study initially pinpointed the microbiome's ability to regulate energy levels and adiposity in the host and paved the way for the study of obese mouse models to determine their efficiency at extracting energy from the diet compared to lean mouse models.

Studies on obese mouse models have further shown how certain gut structures and thus functions impact host regulation of energy and adiposity. The transfer of obese associated microbiota to GF mice results in a significantly greater weight gain than does the transfer of lean associated microbiota to GF mice (Turnbaugh et al., 2006; Turnbaugh and Gordon, 2009; Backhed et al., 2007).

These studies involving mouse models are important because they are able to identify that the determining factor that contributes to weight gain, and by proxy, energy budget, is the composition of their gut microbiome. Thus, the ability to extract either more or less energy from the same diet can be attributed to the metabolic functions of the gut microbes.

While this has implications for fighting obesity in the western world, it is also important for studies on human evolution. From an evolutionary perspective, we can hypothesize how this ability to differentially harvest more calories from food items solely due to the structure (and thus function) of the host's gut microbiome may have been a beneficial characteristic that conveyed fitness benefits in early *Homo*. If the microbiome did convey fitness benefits through increased energy sequestration, it would have been selected for in our core functional microbiome as hominins evolved more metabolically expensive body plans.

Energy Harvesting from Indigestible Dietary Components

The key player for most of human history here is the polysaccharide contained within the human diet which, until recently, contributed to a significant portion of calories in most human diets (Backhed et al., 2005; Cordain et al., 2005). An important way the gut microbes contribute to host digestion and metabolism is by digesting the plant polysaccharide components of the diet that would otherwise be indigestible to the host, and their caloric potential inaccessible. Specific gut microbes have the ability to digest plant-derived polysaccharides that enter into the large intestine mostly untouched, such as pectin, cellulose, hemicellulose, and resistant starches (Backhed et al., 2005). The host gains nutrients and energy from polysaccharide digestion, while the

microbes receive their preferred diet and the oxygen-depleted environment they require (Backhed et al., 2005).

This highlights the need to examine the prevalence of microbial species present in the human gut that are specialized for the processing of dietary polysaccharides. This will indicate to us something about individual variation in energy harvesting ability which will have important implications for our evolution (Backhed et al., 2005).

It is important to note here that these plant-derived components of the human diet are lacking in westernized populations who eat a diet higher in processed foods, meats, and simple carbohydrates, than most other populations on the earth. This is hypothesized to have led to a gut that is in dysbiosis with the human nuclear genome, and that is characterized by decreased microbial diversity and the loss of some taxa and their functions across successive generations (Sonnenburg and Sonnenburg, 2019; Blaser, 2006; Cordain et al., 2005).

How SCFAs are Utilized by the Body and Contribute to the Host Energy budget

There are two ways in which the gut microbiome may impact host energy and physiology: by extracting energy from otherwise indigestible components of the diet or by increasing fat stores/adiposity by impacting the regulation of host genes and metabolism. (Turnbaugh and Gordon, 2009; Backhed et al., 2007). Both pathways can result in increased energy gain for the host and serve as a buffer against periods of low caloric intake.

The indigestible fibers of the host diet will pass through the stomach and small intestine relatively unchanged. Once in the large intestine, however, they are fermented by obligate anaerobes into metabolites predominantly consisting of SCFAs (Den Besten

et al., 2013). This process is important to the host energy budget because the plant-based polysaccharides, as well as oligosaccharides and resistant starches (Den Besten et al., 2013), are fermented to produce short chain fatty acids (SCFAs) such as acetate, propionate, and butyrate (Nieuwdorp et al., 2014). Different fibers will be fermented by specialized microbes into different SCFAs (Den Besten et al., 2013). Bacteroidetes is responsible for producing acetate and propionate and Firmicutes is responsible for producing the majority of butyrate (Macfarlane and Macfarlane, 2003).

The host is then able to absorb these SCFAs, with the majority being taken up by the host and used as energy and the rest excreted in feces (Den Besten et al., 2013). SCFAs can then directly impact metabolism in colonocytes or are transported out of the cecum and colon and into the bloodstream. They are then transported to various organs where they either act as a substrate or signal molecule, impacting lipid, glucose, and cholesterol metabolism. The liver, heart, adipose tissue, kidney, and muscle tissue metabolize available acetate, and, in the liver, propionate is also a precursor molecule for gluconeogenesis (Den Besten et al., 2013).

SCFAs also impact the regulation of adipose tissue. Once in the bloodstream, SCFA concentration can be detected by free fatty acid receptors. These receptors are implicated in regulating the metabolism of lipids and glucose molecules (Den Besten et al., 2013), and they also play a role in appetite control (Flint et al 2008; Sleeth et al., 2010). In this way, SCFAs have the ability to regulate fatty acid synthesis, oxidation, and lipolysis. The result is that free fatty acid concentration in the blood plasma is reduced, as is body weight (Den Besten et al., 2013).

SCFAs are transported to the liver and muscle tissues, where they increase AMPK activity. This in turn triggers further pathways which are important in regulating cholesterol, lipid, and glucose metabolism. This increases fatty acid oxidation in both tissues and decreases fatty acid oxidation in the liver. Lastly, SCFAs have been implicated in increasing thermogenesis and fatty acid oxidation in brown adipose tissue by increasing protein expression of PGC-1 and uncoupling protein-1 (Den Besten et al., 2013).

The ability of the host to extract as much usable energy as possible from their diet will depend on their individual microbiome composition. Depending on the efficiency of their microbes, loosely translated to mean a high abundance of SCFA producing microbes, the host derives up to 10% of their daily caloric requirements through host metabolism of microbe-derived SCFAs. The intestines are an energy hungry organ, and colonocytes can derive up to 70% of their energy from SCFAs (Den Besten et al., 2013; (Bergman, 1990; Serpa et al., 2010). This is a significant portion of the overall energy budget that, as I discuss in the next section, deserves to be analyzed in the context of hominin bioenergetics and evolution.

Today an efficient gut microbiome is associated with an obese phenotype. It may be that humans are predisposed to, or coevolved with, this microbiome structure and function, and thus to obesity when dietary circumstances allow. It is intriguing to view this predisposition as an evolutionary advantage with ancient roots, rather than a modern phenomenon driven solely by (generally) a westernized lifestyle. The evolution of the human's energy-expensive brain and gut may have relied to a certain extent not just on

increasing diet quality but on the ability to efficiently extract calories and energy from the host's diet, which I will explore briefly in the next section.

Chapter 3: The Evolution of Encephalized *Homo* and the Role of the Gut Microbiome

The Encephalized Brain as an Energetically Costly Organ

The previous section gave a thorough discussion on the important ways in which microbes contribute to our energy budget and metabolism. Here, I will begin exploring the ways in which this contribution from the gut microbiome is essential to apply to frameworks of hominin evolution. It will allow for a deeper understanding of how shifting energy budgets over the past four million years of hominin evolution contributed to a highly encephalized brain.

Brains are energetically costly tissues, (Mink et al., 1981) and our large encephalized brains consume roughly 20% of our resting metabolic rate (RMR) (Leonard and Robertson, 1992). In contrast, our closest living relative the chimpanzee, *Pan troglodytes*, has a brain roughly ⅓ the size of our own (Navarrete et al., 2011) that consumes 13% of their RMR (Mink et al., 1981; Holliday, 1986). Despite the differing proportions of energy shunted to the brain at rest, previous estimates of average relative energy consumption rates of both *H. sapiens* and *P. troglodytes* at rest (RMR) have been about equal (Bruhn and Benedict, 1936; Leonard and Robertson, 1992).

This discrepancy between the total RMR and the brain's RMR in humans and chimpanzees has become something of an evolutionary paradox within bioenergetics research (Pontzer et al., 2016). For example, if humans and their ancestors are characterized as having larger brains than expected for mammals of our size that are energetically costly, it may logically follow that our energy intake must be higher than that of our closest living relative (Navarrete et al., 2011).

Under this past research, it was suggested that energy was allocated differentially within the *Pan* and *Homo* lineages, suggesting that the hominin lineage has reallocated energy within their budget to pay for a larger brain. However, more recent research has found a significant difference in RMR between chimpanzees and humans and refutes the original suggestion that RMR is the same across these genera (Pontzer et al., 2016). Research now suggests that humans have in fact evolved an increased BMR in order to support an energetically expensive large brain.

Pontzer et al., suggest this increase in BMR may be paid for by a variety of factors, including increased diet quality, food sharing, and allomaternal care, as well as increased fat deposits (2016). They do note that an increased BMR and TEE may make hominins more susceptible to energy shortfalls and suggest that increased body fat coevolved with an elevated TEE to guard against periods when appropriate caloric intake may be difficult. Humans thus have a predisposition to increased adiposity, in contrast to other hominoids, who remain lean even in settings of lower activity levels.

It is important to note here that efficient microbiomes that extract higher amounts of energy than other structures also contribute to and regulate adiposity. This may serve as further evidence that it is an evolutionary adaptation to help support a higher energy requirement and guard against a potential energy deficit while supporting an encephalized brain.

Early *Homo*

Perhaps one of the most intriguing questions surrounding human evolution that still lacks a sufficient answer is how ancient hominins were able to afford the relatively

large brains that are characteristic of the genus *Homo* (Pontzer et al., 2016; Anton et al., 2014). The appearance of *H. erectus* and its more characteristically human-like morphology has garnered enormous attention within the paleoanthropology community over the years, and researchers have tried to postulate the evolutionary pressures and circumstances that led to this change in hominin morphology and physiology.

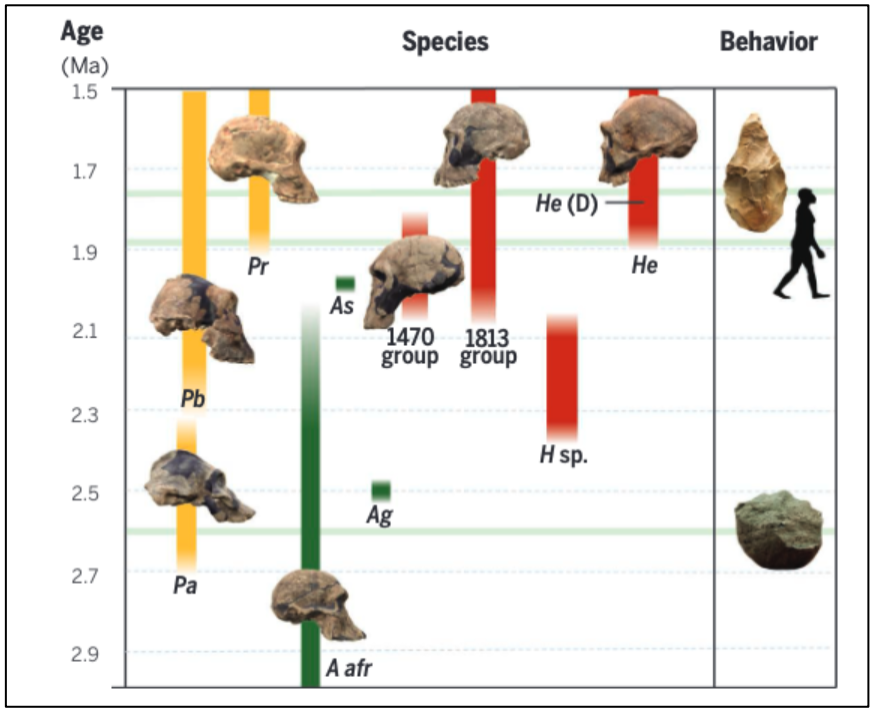

Figure 1. Timeline of early hominin species and the appearance of modern behaviors associated with *Homo* in the fossil record (Anton et al., 2014).

We first see an encephalized brain in the hominin lineage with the appearance of *Homo erectus* in the fossil record at about 2 MA (Figure 1). A number of behaviors appear around this time, such as tool use and an associated increase in diet quality and energy efficient bipedalism. An increase in hominin diet quality is assumed to have accompanied the use of stone tools for butchery and the use of fire (Anton et al, 2014). These traits, or modern behaviors, that accompany the appearance of *H. erectus*

are considered defining characteristics of the genus *Homo* and potentially would have contributed to an increase in energy budget to pay for an encephalized brain.

However, these behaviors don't appear in the archaeological record at the same time, indicating that they were not a package of developmental traits that arose together (Figure 1) (Anton et al, 2014). This then leaves a gap in the record that does not account for how hominins paid for an encephalized brain, which would have required more energy at rest than the brains of earlier Australopiths.

Current Energy Frameworks

A few frameworks exist to model how early *Homo* afforded this larger brain: the Expensive Tissue Hypothesis (Aiello and Wheeler, 1995) and the Expensive Brain framework (Isler and van Schaik, 2009; Navarrete et al., 2011), the latter of which incorporates both a reallocation of energy and an increase in overall net energy. The Expensive Tissue Hypothesis (ETH) was the first hypothesis proposed to account for the high energetic cost of large brains. It suggested a reallocation of hominin energy budgets to prioritize metabolically expensive tissues (the brain) and reduce other high-cost tissue such as the gastrointestinal organs (Aeillo and Wheeler, 1995). Essentially, as hominins began to consume more meat, cooked foods, and tubers (which are high in fiber), they needed less digestive power of a long GI tract, and over time evolved for a smaller digestive tract. The energy saved from the digestion of higher quality food items in a smaller digestive tract allowed for the energy surplus that, over time, contributed to the evolution of a large brain (Aeillo and Wheeler, 1995).

While it is true that modern humans have smaller guts than their ancestors Leonard and Robertson, 1996), more recent literature refutes the ETH as the sole

framework to explain how hominin energy budgets were reallocated. At best, some data support a reduction in gut size compared to brain size (Aiello and Wheeler, 1995; Fish and Lockwood, 2003), especially in anthropoid primates, and other data refute it in other mammals (Jones and MacLarnon, 2004; Isler and van Schaik, 2006a).

Current frameworks aimed at addressing how the necessary energy surplus was achieved suggest that many non-exclusive factors worked together within the evolution of the hominin lineage. Isler and van Schaik (2009) and Navarrete et al., (2011) propose the Expensive Brain Framework, which explores energy contributions to the brain stemming from both an increased net energy input as well as a change in energy allocation (Figure 2) (Navarrete et al., 2011). Navarette et al., (2011) suggest many factors leading to an increased energy budget and includes both a reallocation of energy and an increase in overall net energy (Isler and van Schaik, 2009).

It is within this existing framework that I suggest an additional pathway to be included incorporating the metabolic potential of the gut microbiome (Figure 2). I suggest that the gut microbiome's differing ability to extract host-useable calories from the diet was a contributing factor in creating an energy surplus (Turnbaugh et al., 2006; Turnbaugh and Gordon 2009; Backhed et al., 2004). It is possible that microbes more efficient at extracting energy from food sources would have been selected to help early *Homo* pay for an encephalized brain, and may have been working to influence their energy budget in ways the archaeological record could not capture.

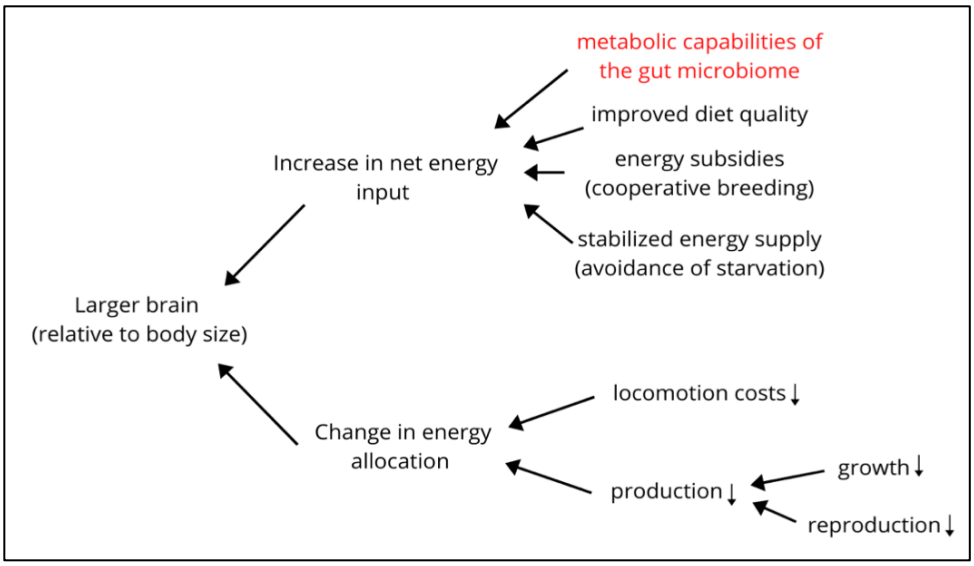

Figure 2. The Expensive Brain Framework modified to include the metabolic capabilities of the gut microbiome (modified from Navarrete et al., 2011).

While the literature discussed in the previous section shows that SCFAs are not delivered to the brain (indeed, the brain prefers only glucose (Leonard and Robertson, 1992)), SCFAs might supplement the energy of other energy-hungry tissues, such as the GI tract and the liver, and thus allow for other energy sources to be redirected to, and prioritized by, the brain. Additionally, a microbiome that can extract more energy may have been favored over a moderately producing microbiome as a buffer against energy shortfalls that Pontzer et al., (2016) assert would have been a risk of increased hominin BMR.

I suggest that the metabolic contributions of the gut microbiome must be incorporated in future studies on bioenergetics and the evolution of the genus *Homo*. This will necessarily include studies on model organisms and comparative primate studies to examine how SCFAs support hominid energy budgets.

Chapter 4: Background on Data Analysis

There is a large gap between the age of microbiomes we can reliably study (any modern samples and up to 2- 3 KA) and the time when encephalized *Homo* first appears in the fossil record (about 2 MA). While we cannot study early *Homo* directly, analyzing more recent samples may reveal more about the complex evolved mutualism between host and microbe.

There are currently limited ways to explore the role of gut microbes in early *Homo*. Studies on mouse models currently remain one of the best ways to study the microbiome and apply findings to theories of mutualistic coevolution (Turnbaugh et al., 2006). Other methods include comparative primate studies from which we can better learn about conserved or derived functions of the gut, and the role of environment and diet in influencing changes in gut structure both over short periods of time and generationally (Amato, 2016).

Lastly, we can analyze what has been called the ancestral gut microbiome of pre-industrial *H. sapiens* via the omics analysis of coprolites (Wibowo et a., 2021). It is thought that the ancestral structure represented by these coprolites is most similar to the one that *H. sapiens* evolved with overtime, prior to the dramatic shifts in lifestyle and diet in the past 200 years that have altered the composition and function of gut structures mainly in people following a westernized diet (Sonnenburg and Sonnenburg, 2019; Blaser, 2006; Cordain et al., 2005).

Some research suggests that this has created a genetic mismatch between the functions of the gut microbes and the host, which has increased the prevalence of non-communicable chronic disease in westernized populations (Sonnenburg and Sonnenburg,

2019; Blaser, 2006; Cordain et al., 2005). Thus, we assume that the western gut may not be its most advantageous form, though some research argues that our guts have evolved accordingly with our environment and are therefore not a detriment to our fitness. Prior to the changes that accompanied the industrialization of the agricultural process and diet shifts in westernized nations (Sonnenburg and Sonnenburg, 2019; Blaser, 2006; Cordain et al., 2005), it would have been evolutionarily favorable to have a gut microbiome that was as efficient as possible at extracting calories from components of the diet that were indigestible by the host.

Coprolites serve as a snapshot in time before these changes and may be useful in inferring what a core functional microbiome would have looked like for *H. sapiens* before dietary change in the past 200 years influenced many populations' diets and, thus, their microbiomes. This can be used for public health research that aims to identify the origination or basis of pathogenesis for a wide variety of diseases and to direct the development of pharmaceutical therapeutics. Additionally, it can be used to learn something more about the energy-producing capacity of the gut for a particular individual through the analysis of microbes present in the coprolites.

Here, I present an original genomics analysis of previously published fecal microbiome data (Wibowo et al., 2021), with the goal of examining which types of microbes are present at differential levels across the samples. While this does not serve as a direct proxy for the guts of *H. erectus*, it will begin to reconstruct a picture of 'ancestral' gut microbiomes and allow for the analysis of their structure and function.

I predict that *H. sapiens* fecal microbiome samples, dating to before the industrialization of the agricultural process and the influx of processed foods in

westernized diets, will have higher relative abundances of SCFA producing microbes in their guts. This higher abundance will likely be a product of coevolution between host and endosymbiont that confers metabolic advantages to the host to support their increased resting metabolic rate and an encephalized brain. I also expect that there will be higher abundances of other microbes that are specialized for digesting fibrous plant polysaccharides in the paleo samples and that these microbes may appear in association with SCFA producing microbes

Chapter 5: Methods

Data Cleaning and Organization

MetaPhlAn output from Wibowo et al., (2021) was compiled into a new datasheet that included information on sample and microbe composition (samples were either collected or collated by Wibowo et al., (2021). The data was organized in a way that kept the samples' identification numbers assigned when they were collected for analysis in their original studies. Samples kept for this analysis corresponded to either coprolite samples (n = 8) or modern human samples (n = 789), and the soil samples (n=3) that had been used for quality control in Wibowo et al., (2021) were excluded.

Data accessed from the supplemental information in Wibowo et al., (2021) was compiled into the metadata and contained information such as the location of the sample and its type, either paleofeces, industrial, or non-industrial. Paleofeces samples reference the ancient coprolites dating to 2 KA. The industrial samples refer to samples from modern individuals living a western lifestyle. Non-industrial samples refer to samples from modern individuals living a subsistence-based lifestyle, which includes both agrarian and hunter-gatherer. Each sample in the meta-data had an identification number, type, and location that corresponded to their identification number in the data.

Data Analysis

MaAslin2 (Multivariate Association with Linear Models 2) (Mallick, et al., 2021) was used to identify differentially abundant taxa across the three categorizations of sample type: paleofeces, industrial, and non-industrial. The MaAslin2 model was fit with

the Data and Meta Data constructed from Wibowo et al. (2021). The paleofecal samples were used as the baseline from which to compare the abundances of microbes.

Data Visualization

omicsEye/omicsArt R package (Rahnavard, 2021) was utilized to produce a Principal Coordinate Analysis for zero-inflated data using the Data and MetaData. omicsArt was also utilized to produce a plot of the Shannon Diversity index, which measured the diversity of species grouped by their sample location. A Shannon Diversity index was also created for samples by their type, either industrial, non-industrial, or paleofeces.

With information from MaAslin2, certain taxa that were significantly differentially abundant between samples were extracted from the Data for further visualization in R, such as creating plots of mean abundance by sample type.

All data manipulation and statistical analyses were performed in R version 4.1.1.

Chapter 6: Results

The PCoA plots that were produced to better visualize the differences between the samples show that the industrial and non-industrial samples group distinctly with other samples from similar dietary lifestyles based on their microbiome composition (Figure 3). The paleofeces appear more similar to the non-industrial samples and map more closely to them. The Shannon Diversity Index (Figure 4) shows that the industrial samples had a higher species diversity compared to the paleofeces and the non-industrial samples.

MaAslin2 identified 108 taxa that were significantly differentially abundant in the industrial and non-industrial samples as compared to the paleofeces (max significance using target FDR = .05). The first two taxa that were the most significantly differentially abundant were both Lachnospiraceae, which had an overall lower abundance in both non-industrial (q-value = $2.092e^{-31}$) (Figure 5) and industrial samples (q- value $3.116e^{-30}$) (Figure 6).

*Ruminococcus champenallensi*s was the next most significantly differentially abundant microbe as expressed by MaAslin2. It had a lower relative abundance in both industrial (q-value =$1.198e^{-18}$) (Figure 7) and non-industrial samples (q-value = $1.789e^{-13}$) (Figure 8) compared to the paleo samples.

The top 50 microbes with significant associations are summarized in the heat map below (Figure 9). Many of the microbes represented here are associated with short chain fatty acid production and the degradation of plant-based fibers and dietary carbohydrates.

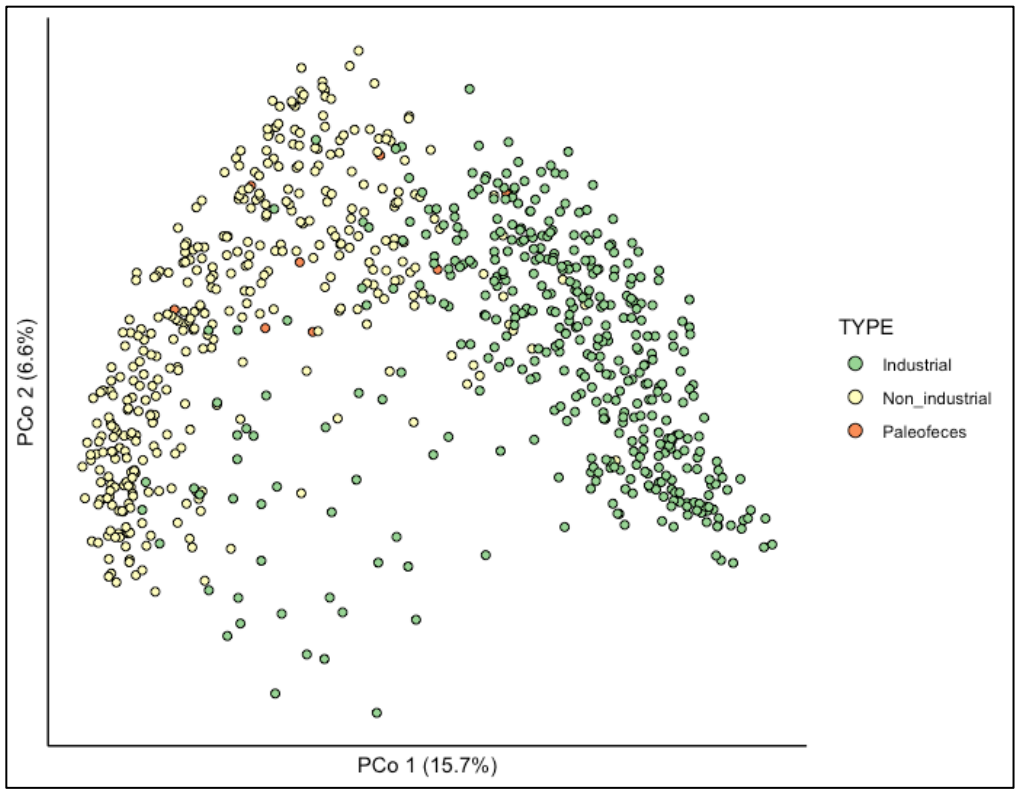

Figure 3. Principal Coordinate Analysis of all samples identified by type.

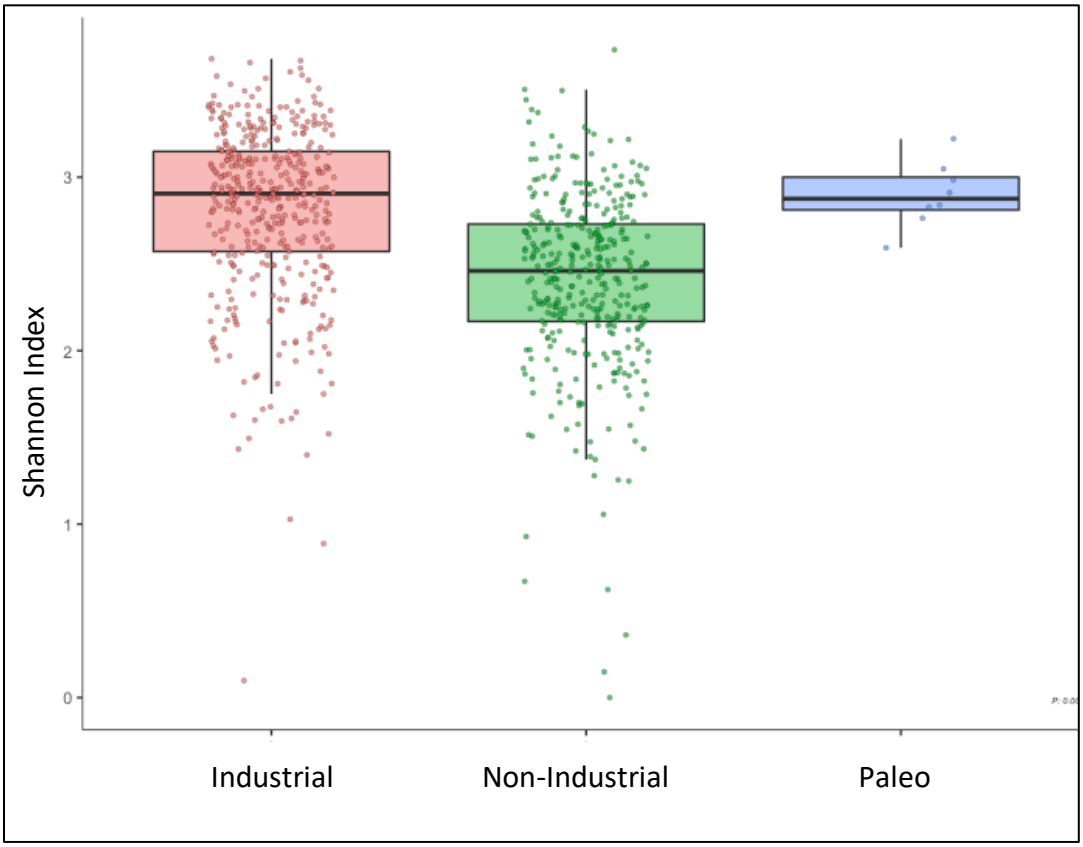

Figure 4. Shannon Index across samples by sample type.

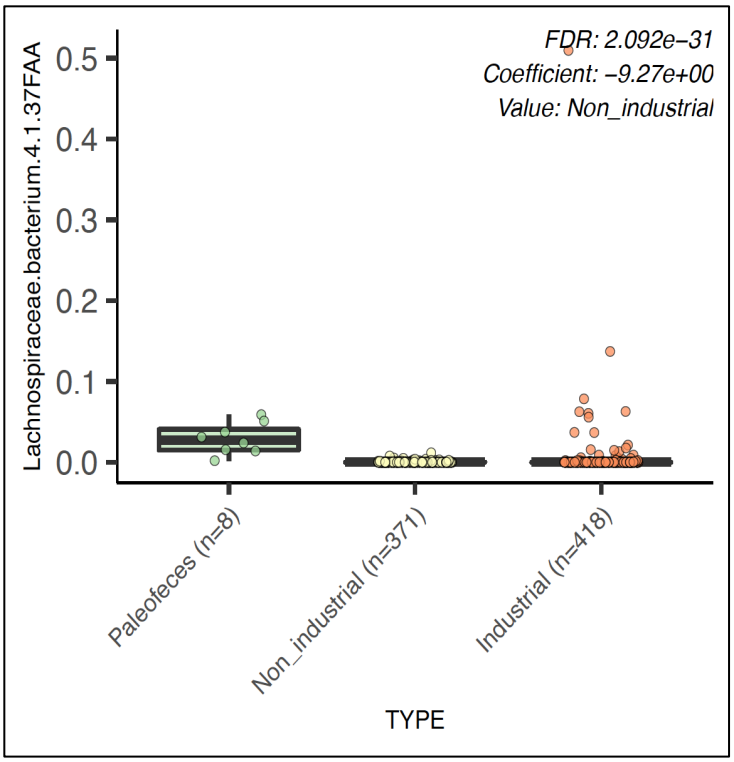

Figure 5. MaAslin2 output of Lachnospiraceae relative abundance across sample type with significance in the non-industrial group.

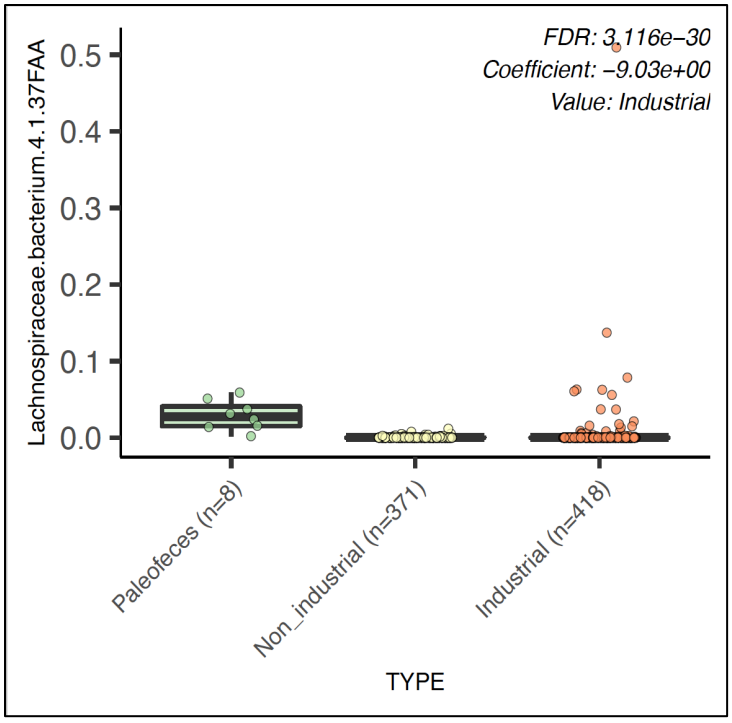

Figure 6. MaAslin2 output of Lachnospiraceae relative abundance across sample type with significance in the industrial group.

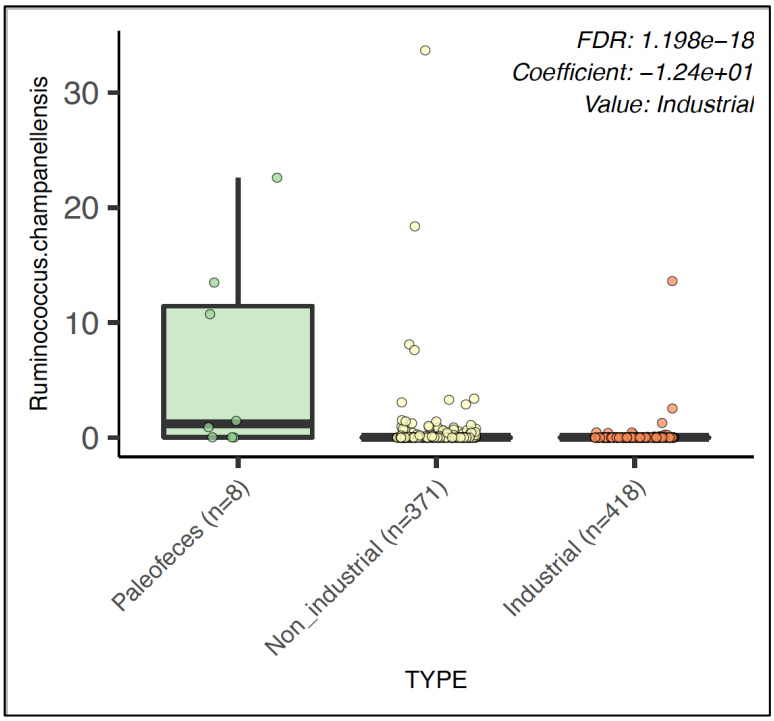

Figure 7. MaAslin2 output of *Ruminococcus champanellensis* relative abundance across sample type with significance in the industrial group.

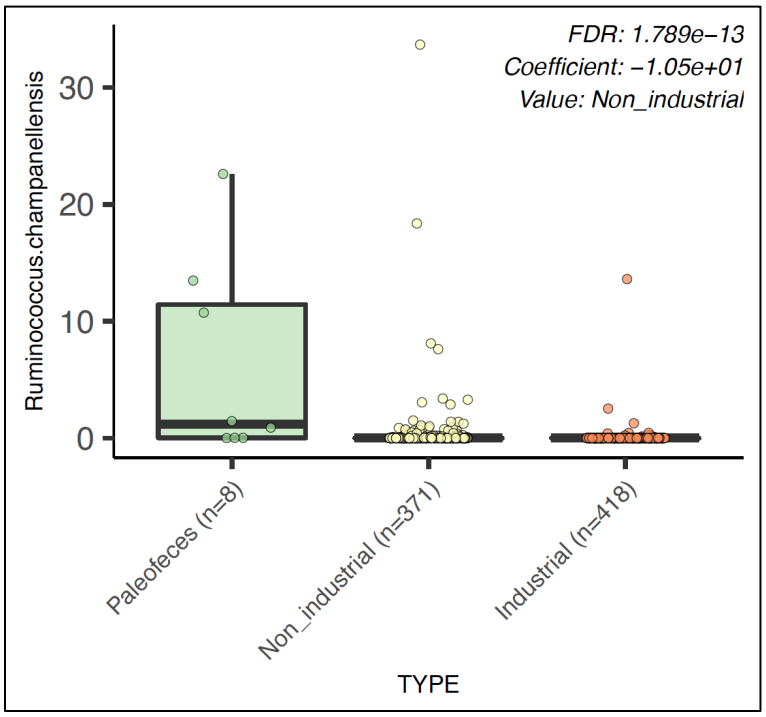

Figure 8. MaAslin2 output of *Ruminococcus champanellensis* relative abundance across sample type with significance in the non-industrial group.

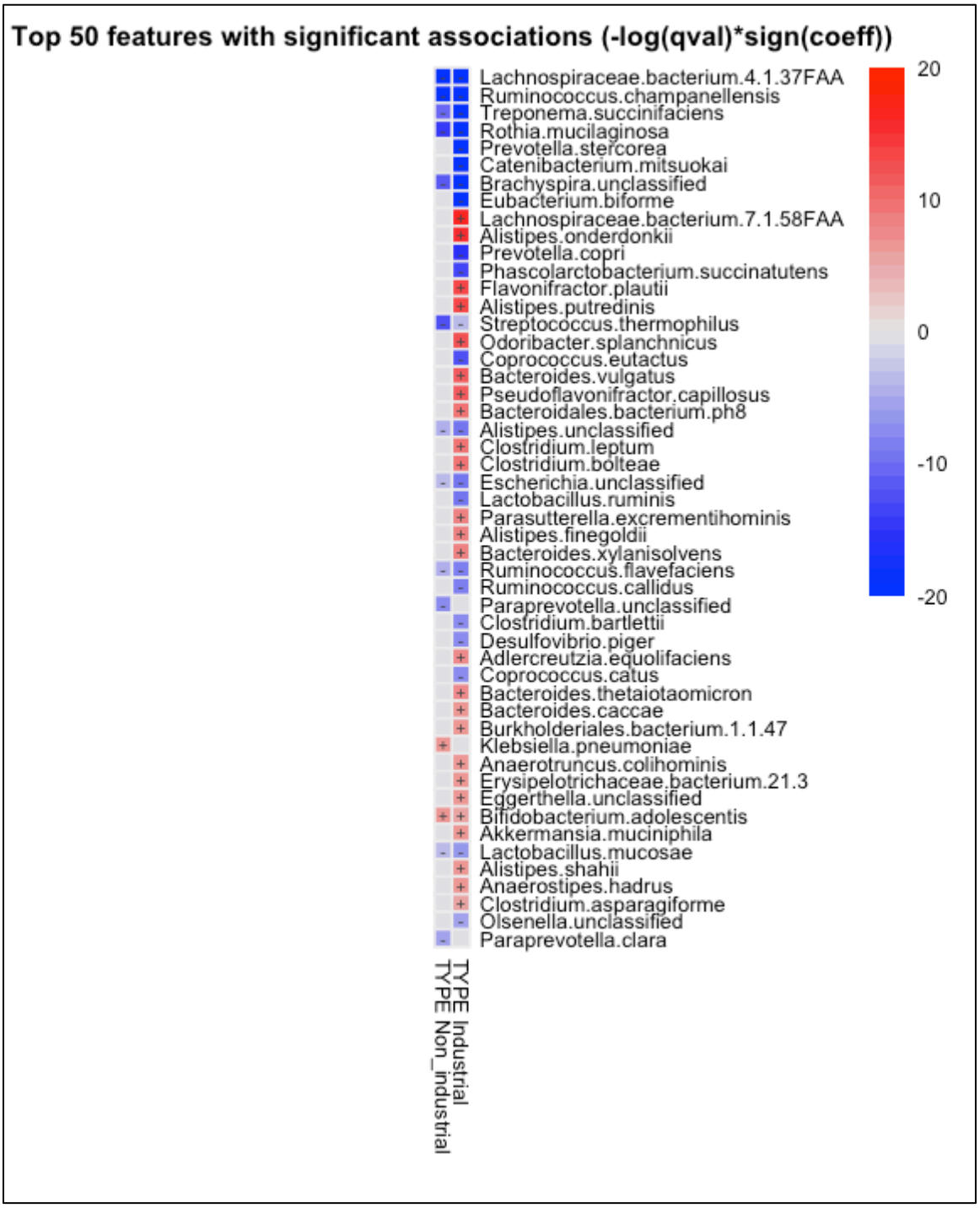

Figure 9. Heat map of significantly associated features with Paleofecal samples as the control.

Chapter 7: Discussion

The paleofecal samples are assumed to represent the ancestral state of the human gut microbiome and are representative of a structure that evolved in context with our nuclear genes. More recent changes to lifestyle and the agricultural industry have increased the pace of change in our gut microbiome, which has outpaced the rate of change of our more slowly evolving nuclear genome. The Principle Coordinate Plot (Figure 3) displays the impact of lifestyle changes on the gut microbiome. There is a clear structural difference between the industrialized samples and the non-industrialized samples. The non-industrial samples correspond with individuals living an agrarian or hunter-gatherer lifestyle who generally eat diets higher in fibrous polysaccharides than individuals in the westernized populations. The paleo samples are representative of peoples who ate a diet higher in plant fibers and date to pre-industrialized times. Their diet was more similar to modern non-industrial populations, which then follows that they map more similarly onto the non-industrial samples.

This expected finding contrasts with the diversity index displayed in the plot of the Shannon Diversity Index (Figure 4). This plot shows that, across sample types, the non-industrial samples had a lower species diversity than the industrial or paleo samples. This result was unexpected since individuals eating diets higher in plant-based polysaccharides are generally higher in diversity than westernized populations.

To study the structure and function of the human gut microbiome prior to the dietary changes seen in westernized samples, paleo samples serve as the best proxy. By studying paleo samples, we can begin to more deeply explore the evolutionary relationship between host and gut microbe that results in a complex mutualism.

Lachnospiraceae is a bacteria family in phylum Firmicutes and the class Clostridia, and is related to short chain fatty acid production, specifically butyrate (Meehan and Beiko, 2014). It is notable that MaAslin2 identified this microbe as being significantly differentially abundant in the industrial and non-industrial samples compared to the paleo samples. While there are other microbes found in the human gut specialized for SCFA production, it is important that we see differences in Lachnospiraceae abundance across samples types, since this may indicate to us that the functions of this microbe were previously selected for in the human gut. If ancient *Homo* evolved with the selection of metabolically efficient gut microbes, this would be an expected conserved function across hominin descendants. These descendants, including the paleo samples, may then have continued to rely on these endosymbiont mutualisms. We would thus expect to see ancient gut structures with a relative abundance of SCFA producing microbes significantly higher than in modern humans who have diets and/or lifestyles greatly altered from our 'ancestral' way of life.

The paleo samples also had a higher relative abundance of microbes related to plant polysaccharide digestion. The structure of the microbes themselves is influenced by dietary composition, and so the paleo samples may represent a gut structure more specialized for digestion of an ancestral diet. The microbial ability to digest host diets and to produce SCFAs in ancient human guts may be important related processes whose contributions to our physiology influenced our evolution. It is thus significant that we see these microbes in higher relative abundance in the paleo samples than in either of the modern ones.

Chapter 8: Conclusions

The biomarker discovery analysis of the ancient paleofecal samples identifies candidate microbes that will be important to analyze in future studies. It will be important to quantify how Lachnospiraceae and other SCFA producing microbes contribute to the host's overall energy supply in different diets, and if this may be a significant contribution that has been selected for during the evolutionary history of *Homo*. Energetics studies that work to determine how the microbiome contributes to the energy economy of *H. sapiens* today and in more recent ancient times will be essential in hypothesizing the role gut microbes may have played in early *Homo* evolution. The metabolic capabilities of gut microbes must be incorporated into current and future frameworks on the evolution of encephalized brains in hominins, since their contribution to humans today is significant and essential to our physiology. It is possible SCFA producing microbes have been selected over time and may be responsible for our modern body plan in ways that the archaeological record has failed to record.

Bibliography

Abubucker, S., Segata, N., Goll, J., Schubert, A. M., Izard, J., Cantarel, B. L., ... & Huttenhower, C. (2012). Metabolic reconstruction for metagenomic data and its application to the human microbiome. *PLoS computational biology*, *8*(6), e1002358.

Aiello, L. C., & Wheeler, P. (1995). The expensive-tissue hypothesis: the brain and the digestive system in human and primate evolution. *Current anthropology*, *36*(2), 199-221.

Amato, K. R. (2016). Incorporating the gut microbiota into models of human and non-human primate ecology and evolution. *American Journal of Physical Anthropology*, *159*, 196-215.

Antón, S. C., Potts, R., & Aiello, L. C. (2014). Evolution of early Homo: An integrated biological perspective. *science*, *345*(6192), 1236828.

Bäckhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., ... & Gordon, J. I. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the national academy of sciences*, *101*(44), 15718-15723.

Backhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A., & Gordon, J. I. (2005). Host-bacterial mutualism in the human intestine. *science*, *307*(5717), 1915-1920.

Bäckhed, F., Manchester, J. K., Semenkovich, C. F., & Gordon, J. I. (2007). Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proceedings of the National Academy of Sciences*, *104*(3), 979-984.

Bäckhed, F., Fraser, C. M., Ringel, Y., Sanders, M. E., Sartor, R. B., Sherman, P. M., ... & Finlay, B. B. (2012). Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications. *Cell host & microbe*, *12*(5), 611-622.

Bergman, E. N. (1990). Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological reviews*, *70*(2), 567-590.

Blaser, M. J. (2006). Who are we? Indigenous microbes and the ecology of human diseases. *EMBO reports*, *7*(10), 956-960.

Bruhn, J. M., & Benedict, F. G. (1936). The respiratory metabolism of the chimpanzee. In *Proceedings of the American Academy of Arts and Sciences* (Vol. 71, No. 5, pp. 259-326). American Academy of Arts & Sciences.

Cordain, L., Eaton, S. B., Sebastian, A., Mann, N., Lindeberg, S., Watkins, B. A., ... & Brand-Miller, J. (2005). Origins and evolution of the Western diet: health implications for the 21st century. *The American journal of clinical nutrition*, *81*(2), 341-354.

Davenport, E. R., Sanders, J. G., Song, S. J., Amato, K. R., Clark, A. G., & Knight, R. (2017). The human microbiome in evolution. *BMC biology*, *15*(1), 1-12.

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., ... & Turnbaugh, P. J. (2014). Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, *505*(7484), 559-563.

Den Besten, G., Van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D. J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of lipid research*, *54*(9), 2325-2340.

Fish, J. L., & Lockwood, C. A. (2003). Dietary constraints on encephalization in primates. *American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists*, *120*(2), 171-181.

Flint, H. J., Bayer, E. A., Rincon, M. T., Lamed, R. & White, B. A. (2008). Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nat. Rev. Microbiol.* 6, 121–131.

Gao, Z., Yin, J., Zhang, J., Ward, R. E., Martin, R. J., Lefevre, M., ... & Ye, J. (2009). Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes*, *58*(7), 1509-1517.

Holliday, M. A. (1986). Body composition and energy needs during growth. In *Postnatal growth neurobiology* (pp. 101-117). Springer, Boston, MA.

Isler, K., van Schaik, C.P., (2006a). Costs of encephalisation: the energy trade-off hypothesis tested on birds. J. Hum. Evol. 51, 228–243.

Isler, K., & van Schaik, C. P. (2009). The expensive brain: a framework for explaining evolutionary changes in brain size. *Journal of human evolution*, *57*(4), 392-400.

Jones, K.E., MacLarnon, A.M. (2004). Affording larger brains: Testing hypotheses of mammalian brain evolution on bats. Am. Nat. 164, E20–E31.

Leonard, W. R., & Robertson, M. L. (1992). Nutritional requirements and human evolution: a bioenergetics model. *American Journal of Human Biology*, *4*(2), 179-195.

Leonard, W. R., Robertson, M. L. (1996). On diet, energy metabolism, and brain size in human evolution. *Current Anthropology*, *37*(1), 125-129.

Ley, R. E., Lozupone, C. A., Hamady, M., Knight, R., & Gordon, J. I. (2008). Worlds within worlds: evolution of the vertebrate gut microbiota. *Nature Reviews Microbiology*, *6*(10), 776-788.

Lloyd-Price, J., Mahurkar, A., Rahnavard, G., Crabtree, J., Orvis, J., Hall, A. B., ... & Huttenhower, C. (2017). Strains, functions and dynamics in the expanded Human Microbiome Project. *Nature*, *550*(7674), 61-66.

Macfarlane, S., & Macfarlane, G. T. (2003). Regulation of short-chain fatty acid production. *Proceedings of the Nutrition Society*, *62*(1), 67-72.

Mallick H, Rahnavard A, McIver LJ, Ma S, Zhang Y, Nguyen LH, Tickle TL, Weingart G, Ren B, Schwager EH, Chatterjee S, Thompson KN, Wilkinson JE, Subramanian A, Lu Y, Waldron L, Paulson JN, Franzosa EA, Bravo HC, Huttenhower C. (2021). Multivariable Association Discovery in Population-scale Meta-omics Studies. PLoS Computational Biology, 17(11):e1009442.

Meehan, C. J., & Beiko, R. G. (2014). A phylogenomic view of ecological specialization in the Lachnospiraceae, a family of digestive tract-associated bacteria. *Genome biology and evolution*, *6*(3), 703-713.

Mink, J. W., Blumenschine, R. J., & Adams, D. B. (1981). Ratio of central nervous system to body metabolism in vertebrates: its constancy and functional basis. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *241*(3), R203-R212.

Navarrete, A., Van Schaik, C. P., & Isler, K. (2011). Energetics and the evolution of human brain size. *Nature*, *480*(7375), 91-93.

Nieuwdorp, M., Gilijamse, P. W., Pai, N., & Kaplan, L. M. (2014). Role of the microbiome in energy regulation and metabolism. *Gastroenterology*, *146*(6), 1525-1533.

O'Hara, A. M., & Shanahan, F. (2006). The gut flora as a forgotten organ. *EMBO reports*, *7*(7), 688-693.

Pontzer, H., Brown, M. H., Raichlen, D. A., Dunsworth, H., Hare, B., Walker, K., ... & Ross, S. R. (2016). Metabolic acceleration and the evolution of human brain size and life history. *Nature*, *533*(7603), 390-392.

Rahnavard, A. (2021). *omicsArt: omics pattern discovery by visualization*. Version 1.0.0.0, https://github.com/omicsEye/omicsArt.

Serpa, J., Caiado, F., Carvalho, T., Torre, C., Gonçalves, L. G., Casalou, C., ... & Dias, S. (2010). Butyrate-rich colonic microenvironment is a relevant selection factor for metabolically adapted tumor cells. *Journal of Biological Chemistry*, *285*(50), 39211-39223.

Sleeth, M. L., Thompson, E. L., Ford, H. E., Zac-Varghese, S. E., & Frost, G. (2010). Free fatty acid receptor 2 and nutrient sensing: a proposed role for fibre, fermentable carbohydrates and short-chain fatty acids in appetite regulation. *Nutrition research reviews*, *23*(1), 135-145.

Sonnenburg, E. D., & Sonnenburg, J. L. (2019). The ancestral and industrialized gut microbiota and implications for human health. *Nature Reviews Microbiology*, *17*(6), 383-390.

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *nature*, *444*(7122), 1027-1031.

Turnbaugh, P. J., & Gordon, J. I. (2009). The core gut microbiome, energy balance and obesity. *The Journal of physiology*, *587*(17), 4153-4158.

Wibowo, M. C., Yang, Z., Borry, M., Hübner, A., Huang, K. D., Tierney, B. T., ... & Kostic, A. D. (2021). Reconstruction of ancient microbial genomes from the human gut. *Nature*, *594*(7862), 234-239.