Exploring Human Biological Variation in Metabolic Hormones

by

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ABSTRACT

The current understanding of the extent of human biological variation is largely limited in scope. Up until now, most of the research has been conducted in largely sedentary, industrialized populations. Recently however, several foundational studies have demonstrated that these populations only represent one notch on the complete spectrum of human variation. As chronic diseases continue to rise worldwide, it is necessary for research to pivot its lens towards understanding the complete extent of human biological variation and the ecological, cultural, and psychological factors that influence its expression. This dissertation expands knowledge on human variation by examining the relationships between two metabolic hormones (leptin and adiponectin) and several health conditions among the Tsimane, a physically active population of forager-horticulturalists in lowland Bolivia that also face significant parasitic and pathogenic stressors. Leptin and adiponectin are key indicators of energy availability and have well-documented associations with certain health conditions in the literature. However, they have been virtually unstudied outside of the typical urban center research contexts. First, I examined the relationship between leptin and adiponectin and their association with both food insecurity and depression. Secondly, I examined the associations between leptin and adiponectin and several indicators of cardiovascular disease. Lastly, I performed laboratory validations to assess the potential limitations of using a relatively new and considerably cheaper option for biomarker analysis (dried blood spot sampling). These studies found associations between the metabolic hormones and food insecurity, depression, and several cardiovascular health indicators. However, several associations deviated from what had been reported in urban settings,

demonstrating the value of exploring human variation outside of typical research contexts. Additionally, this study found that dried blood spot sampling is a very stable alternative to the more expensive and more cumbersome methods of blood collection, even despite location or equipment accessibility for non-local researchers. This opens up an avenue for future researchers to conduct studies that appreciate the extent of human variation without being hindered by cost, travel, and infrastructure.

For my dear friend and greatest supporter, Sebastián Ramírez Amaya (1991-2022).

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CHAPTER 1

Humanity is occupying a unique moment in history, where now more than ever before, there is global connection of people experienced through media and technology. This globalization movement has begun to shed light on what it means to be human in every shape and form. However, in doing so, it has also revealed the very limited knowledge available on the true extent of human variation and has highlighted just how human biology research has failed thus far in its attempts to understand what should be considered "normal". Most of the knowledge that exists about human biology and health is the product of research conducted in Western, educated, industrialized, rich, and democratic (WEIRD) populations (Henrich et al., 2010; Gurven & Lieberman, 2020). Even further, much of that research focused on white research participants from within those populations (Clancy & Davis, 2019). That research was then mapped onto every living human population and any deviations from that "normal" were considered problematic.

The most compelling example of this can best be seen in the extensive use of the body mass index (BMI) in clinical practices worldwide. The guidelines for BMI cutoffs were based on the 1995 World Health Organization report and have since remained unaltered. This is in spite of the many research articles which have demonstrated how BMI cutoffs are not appropriate for diverse racial and ethnic populations and are especially problematic when BMI is used to determine disease risk in these populations (see Stommel & Schoenborn, 2012; Hruschka et al., 2013; 2014). Worse yet, once "normal" has been set at some population baseline, it can then be used to prevent treatment of some sections of the population- the classic example of this is using "race"

in calculating estimated glomular filtration rate (eGFR). The equation to calculate eGFR has a modifier if the patient is African American, thereby giving them approximately a 16-21% lower eGFR, which disqualifies them from getting a diagnosis of high eGFR, which thus prevents them from seeing kidney specialists, or even getting kidney transplants (Vilson et al., 2021; Diao et al., 2021). It is estimated that removing the racial modifier would increase the diagnosis of chronic kidney disease by 20% in African Americans and reclassify what stage of kidney disease for nearly 30% of patients (Vilson et al., 2021; Diao et al., 2021).

Research on contemporary foraging populations has revolutionized the understanding of human health, demography, socioecology, and life history. Though no single population cannot be viewed as direct models of human evolution, contemporary foraging populations do shed light on how certain aspects of human biology and culture vary when combined with variable access to industrial amenities (e.g., health care, sanitation, and technology). Many studies have demonstrated that variation in ecology, subsistence, population history, culture, parasite/pathogen loads, and discrimination can influence human physiology (Ellison, 2017; Gurven & Lieberman, 2020; Konner & Worthman, 1980; Kaplan et al., 2017; Trumble & Finch, 2019). Researchers have instead shifted their focus onto understanding the extent of human variation and what variables influence such variation (Trumble and Schneider-Crease 2020).

Most of the pioneering research was conducted among the Ju/'hoansi (Dobe! Kung) of Namibia and Botswana (Howell, 1979), the Hadza of Tanzania (Blurton Jones, 2016), the Ache of Paraguay (Hill & Hurtado, 1995), and more recently the Tsimane of Bolivia (Gurven et al., 2017). This dissertation project will expand on the knowledge of

human biological variation by exploring the relationship between two metabolic hormones (leptin and adiponectin) and various health outcomes in the Tsimane of Bolivia. Additionally, it will speak to some of the methodological challenges experienced by researchers operating in unfamiliar, and thus difficult areas.

The Tsimane

The Tsimane are an indigenous forager-horticulturalist group occupying the tropical lowlands of Bolivia. They are considered one of the most isolated indigenous groups living in Bolivia. The Tsimane primarily subsist on slash-and-burn horticulture, fishing, hunting, and seasonal gathering. They produce mainly plantains, rice, corn, and sweet manioc. They hunt a wide variety of neotropical mammals and fish in the local rivers, lagoons, and streams. They seasonally gather fruits and other foods, such as nuts and honey. The Tsimane occupy about 90 villages, each ranging from 50 to 500 individuals. These villages are settled along the Maniqui, Quiquibey, and Mato rivers. Their current overall population size is estimated to be about 17,000 Tsimane, with a population growth rate of over 3.5% (Gurven et al., 2017). The modal age at death for the Tsimane is 70 years (SD =6.3). This is similar to other hunter-gatherer and horticulturalist populations (Gurven et al., 2007).

Much of the research that has been done in collaboration with the Tsimane has been associated with the Tsimane Health and Life History Project (THLHP), codirected by Hillard Kaplan, Michael Gurven, Jonathan Stieglitz, and Ben Trumble. The project has three ultimate aims: 1) to understand the bidirectional connections between life history and social behavior in a high fertility, kin-based traditional context, 2) to understand how high pathogen burden influences health and well-being across the life

course and, 3) to understand how industrialization shapes human life histories and sociality. Within the last 10-15 years, road and river access have improved due to development projects, logging, and new technologies. This has allowed researchers to more easily interact with the Tsimane and has additionally caused the Tsimane to have greater access to markets (Gurven et al., 2017).

A 2017 study found that the Tsimane have the lowest reported levels of coronary artery disease of any population recorded to date (Kaplan et al., 2017). The Tsimane's physically active lifestyle and diet are often credited with the low prevalence of coronary artery disease risk factors, such as coronary artery calcium (CAC) levels, cholesterol levels, and blood lipid levels. In the Tsimane, prevalence of high blood pressure, high cholesterol, and glucose concentrations were low among all age groups. In terms of cholesterol specifically, the Tsimane exhibit low total and low-density lipoproteins (LDL) cholesterol levels. Additionally, smoking prevalence is also low. Furthermore, no differences were observed for hypertension, diabetes, BMI >30 kg/m^2, or hypercholesterolaemia across all age categories (Kaplan et al., 2017).

Prevalence of coronary artery atherosclerosis was measured using CAC scoring. 85% of the Tsimane had absolutely no CAC, while 13% had CAC scores of less than 100 and only 3% had CAC scores of greater than 100 (a CAC score over 100 units is considered moderate cardiovascular risk). These low levels of CAC extend into older age individuals. Tsimane men have higher CAC scores than Tsimane women, but Tsimane men still had lower CAC scores in comparison to Japanese women who were previously considered to have the lowest CAC scores for any ethnicity. Factors associated with CAC scores of greater than 0 in the Tsimane were age, body-fat percentage, hs-CRP levels, and

erythrocyte sedimentation rate. In contrast, younger age, female sex, and lower triglyceride levels were more greatly associated with CAC absence (Kaplan et al., 2017).

The mechanisms that protect the Tsimane from CAC development remain unclear. There are several potential explanations that exist such as their subsistence lifestyle (diet and energy expenditure), genetics, inflammation, and immune regulation. The low LDL or a low LDL-to-high density lipoprotein (HDL) ratio seen in the Tsimane may potentially have atheroprotective effects which may be further augmented by the Tsimane lifestyle. Though genetics do play a minor role in the development of coronary artery disease, the extent of its effect among the Tsimane remains unclear. However the increasing cholesterol levels seen in recent years among the Tsimane suggest that they are not likely genetically protected against heart disease (Kaplan et al., 2017). While there is some evidence that atherosclerosis is an inflammatory disease, a high inflammatory burden has been observed with a low prevalence of CAC for all inflammatory markers in the Tsimane. This suggests that in populations with high pathogen burdens and chronic inflammation, atherosclerosis may not be associated with inflammation, particularly when combined with low LDL concentrations (Gurven et al., 2009; Kaplan et al., 2017).

The diet of the Tsimane is of particular interest because the low fat, low sugar, high protein diets are often credited with the robust cardiometabolic health seen in foraging populations (Kraft et al., 2018) and the Tsimane have the lowest levels of cardiovascular disease than any other population (Kaplan et al., 2017). However, the Tsimane are undergoing a crucial nutritional transition that includes increasing access to market foods, such as sugar, lard, oil, and salt. Tsimane consumption of these food

additives increased significantly between 2010 and 2015. Body mass index (BMI), body fat percentage, and proportion of Tsimane individuals who have higher BMIs (>25 kg/m^2 and >30 kg/m^2) have been increasing over time. Between 2010 and 2015, total energy intake and carbohydrate intake increased significantly, but not protein or fat intake (Kraft et al., 2018).

The Tsimane get most of their food through hunting, gathering, fishing, and horticulture. They cultivate small amounts of plantains, corn, rice, and sweet manioc, and they regularly hunt for meat and fish (Gurven et al., 2006). More specifically, their diet consists primarily of 26 types of cultigens, 43 species of fish, 32 species of wild game, some meat from domesticated animals, 15 varieties of wild vegetables and fruits, and 17 types of market foods. Their preferred wild game is mainly collared peccary, paca, coatimundi, gray brocket deer, tapir, and howler monkeys. The market foods are primarily pasta, wheat flour, bred, and sugar (Kraft et al., 2018).

The Tsimane diet is characterized as high energy, with the average individual consuming between 2,422-2,736 calories per day, which exceeds the average caloric intake of Americans when controlling for age, sex, and individual. Only 5% of Tsimane experience severe caloric shortfalls (<1000 kcal/d). The Tsimane also consume high amounts of protein (119-139 grams per day) and high amounts of carbohydrates (376-423 grams per day). In contrast, their diet is relatively low in dietary fat (40-46 grams per day), which is about 50% lower than the average American diet. A majority of their daily calorie intake (64%) is derived from complex carbohydrates. The Tsimane consume higher amounts of key micronutrients (e.g., magnesium, potassium, and selenium) and also consume 1.5-2 times more dietary fiber than Americans. However, their diets have

less sodium, calcium, and vitamins E, D, and K in comparison to Americans. Additionally, the Tsimane have lower dietary diversity in comparison to the average diet in the United States. Despite the fact that Tsimane report 152 distinct types of foods in their dietary recalls, studies have found that only 9 items make up 75% of the Tsimane diet (Kraft et al., 2018).

The Tsimane have higher resting metabolic rates (RMR) and total energy expenditure (TEE) than people in more sedentary, industrialized societies. Tsimane RMR is estimated to be 18-47% higher in women and 22-40% in men when compared to Western populations and Tsimane TEE is similarly elevated. This may be associated with higher levels of leukocytes and helminths, suggesting that high pathogen burdens may contribute to higher metabolic costs (Gurven et al., 2016). Tsimane adulthood is characterized by high levels of physical activity. Tsimane spend roughly 5-6 hours a day in light to moderate activity and very little time is spent sedentary. In men, activity levels peak in the late twenties, then declines thereafter. Activity levels among females remains constant following their early teenage years. Despite the fact that Tsimane are quite active, their activity levels are consistent with other subsistence populations and are not outside the range of normal for developed populations. The research on Tsimane physical activity suggests that the lack of cardiovascular disease observed in the Tsimane cannot be the result of physical activity alone (Gurven et al., 2013).

Throughout development, hunting-related information is shared and taught to Tsimane youth. However, upon achieving adult body size, Tsimane still need 5-15 additional years of hunting experience to become highly skilled hunters. By their fourth decade of life, their physical strength begins to decline continuously (Gurven et al.,

2006). By 60 years of age, more than 60% of Tsimane complain about hearing loss, trouble seeing close distances, difficulties chopping trees, walking long distances, and frequently complain about arthritic pain in their legs, hips, and backs. Most men over the age of 70 can no longer hunt. These functional disabilities are strong predictors of depression among the Tsimane (Stieglitz et al., 2014). Levels of depression among the Tsimane increase with age, as disability begins to limit productivity and sharing ability (Weiss et al., 2012).

Amazonian populations tend to be exposed to a wide variety of pathogens and parasites, including bacterial, fungal, protozoal, and helminth infections (Blackwell et al., 2016). The THLHP frequently diagnose the Tsimane with a variety of infections during their annual clinical exams, including respiratory (20-30%), gastrointestinal (10-30%), and skin (5%) infections (Gurven et al., 2012). The Tsimane are 10 times more likely than Americans to have elevated white blood cell counts. Additionally, their white blood cell levels decline with age, particularly the lymphocytes and eosinophils. This suggests an increase in maintenance costs, because older adults are just as likely to experience infection as their younger counterparts (Gurven et al., 2009). At any given time, up to 70% of the Tsimane are likely to have an infection with helminths (Blackwell et al., 2010; 2016). When compared to most industrialized reference populations, the Tsimane have elevated levels of many immunological markers such as immunoglobulins, eosinophils, erythrocyte sedimentation rate (ESR), B-cells, and natural killer cells (Blackwell et al., 2016).

There has been a considerable amount of work done on the potential implications for the Tsimane living in high parasite, high pathogen environments (see Blackwell et al.,

2010; Gurven et al., 2016; McDade et al., 2008; Trumble et al., 2017; Garcia et al., 2021). This work suggests that immune function is intimately related to other aspects of health, including cardiovascular disease risk, metabolic health, and cognitive function. For example, the Tsimane have notably high resting metabolic rates (RMR) and total energy expenditure (TEE). RMR reflects the energetic costs of homeostasis and often accounts for up to 75% of TEE. Much of the variability seen in RMR is a reflection of physical activity and lean body mass. However, research on the Tsimane suggests that prolonged immune activation from infection may be associated with higher RMR and TEE. Specifically, elevated leukocytes and helminths are associated with higher RMR in the Tsimane. Ultimately, this suggests that high pathogen burden may lead to higher metabolic costs (Gurven et al., 2016).

Additionally, the association between greater inflammation and atherosclerosis and cardiovascular disease risk has been explored in the Tsimane. In industrial populations, inflammation is implicated in arterial aging, atherogenesis, and hypertension, but this relationship has not been well explored outside of the industrial world. As expected, markers of inflammation and infection are much higher in the Tsimane, whereas their HDL cholesterol levels are considerably lower than what is observed in Americans. Research by Gurven and colleagues (2009) found that inflammation was not associated with CVD risk in the Tsimane. Instead, this suggests that inflammation is not a risk factor for CVD and artherosclerosis in and of itself and may likely be offset by healthy metabolism, active lifestyles, smaller body mass, lean diets, lower blood lipid levels, and better cardiorespiratory health (Gurven et al., 2009).

Metabolic Hormones

This dissertation project utilized two key metabolic hormones: leptin and adiponectin. Both these hormones play crucial roles in the signaling of energy availability and energetic allocation and thus are crucial in the maintenance of physiological homeostasis.

Leptin is a 16KDa protein of 167 amino acids, and is a product of the *ob* gene, located on chromosome 7 in humans (Zhang et al., 1994). Leptin, otherwise known as the 'satiety hormone', is synthesized and secreted primarily by white adipose tissue (Zhang et al., 1994). However, leptin can also be produced by many organs, including the heart, vascular smooth muscle, placental tissue, digestive epithelia, the pancreas, and the lungs (Hou & Lou, 2011; Malli et al, 2010; Larsson & Ahren, 1996). Adult leptin levels are associated with body fat percentage and age. Leptin production is primarily regulated by food intake and thus, the amount of circulating leptin is expected to be proportional to the amount of fat tissue in the body (Flier, 1998). Therefore, leptin's crucial role is that it conveys information to the hypothalamus regarding the amount of energy stored in fat (Meier & Gressner, 2004).

Upon its discovery, leptin was thought to be a potential cure for clinical excess weight due to its metabolic functions. This was based on research that showed that *ob/ob* mice have a single base pair mutation in the leptin gene that leads to dysfunctional leptin, increased body weight, low resting metabolic rate, and impaired homeostasis. When these mice were administered exogenous leptin, the phenotype was reversed (Pelleymounter et al., 2005). Additionally, studies suggested that leptin may cross the blood brain barrier to engage hypothalamic effects that serve to suppress

appetite and enhance energy expenditure (Rajala & Scherer, 2003). Many researchers thought that leptin may be the body's natural way of preventing excess weight gain by signaling satiety and reducing overconsumption. Therefore, it would be expected that individuals who were diagnosed as clinically overweight would have inadequate leptin levels and the administration of leptin could potentially be useful in weight reduction (Tena-Sempere, 2007). However, the relationship between leptin and body fat is more complex.

If energy input and output are balanced, then leptin should reflect the proportion of adipose tissue in the body (Frayn et al., 2003). However, leptin concentrations are actually found to be elevated in individuals with a BMI greater than 30 kg/m^2 (Rosicka et al., 2003). Studies that administered recombinant leptin to individuals showed only limited effects on weight loss, regardless of BMI status (Heymsfield et al., 1999). Ultimately, it was determined that this was the result of leptin resistance, which is the desensitization of the leptin signal. This may occur on two levels. Firstly, this resistance may be the product of the saturable transport of leptin across the blood brain barrier. Secondly, it could be due to abnormalities in the extent of leptin receptor activation or signal transduction (El-Haschimi et al., 2000). The result is that individuals with elevated leptin levels experience no decreases in appetite (Frederich et al., 1995). This study supports the notion that leptin likely plays a stronger role in starvation prevention than it does in satiety, which is consistent with what is known about the evolution of human energetic metabolism.

Originally, leptin concentrations were not believed to vary in associating with eating behavior (Meier & Gressner, 2004). However, studies show that leptin production

occurs after insulin increases in association with meal consumption. Therefore, eating behavior and insulin production acutely affect leptin concentrations in the body (French & Castiglione, 2002).

Leptin has been shown to decrease in response to food deprivation. Consequently, leptin may play a strong role in the starvation induced suppression of the hypothalamic-pitutary-gonadal (HPG) axis (Veniant & LeBel, 2003). Individuals who engage in severe dietary restriction (e.g., anorexia nervosa or bulimia nervosa) are likely to have low levels of leptin (Bluher & Mantzoros, 2004). It is well-demonstrated that individuals engaging in caloric restriction and intense exercise are at an increased risk of hypothalamic amenorrhea. It has also been suggested that low leptin levels may play a role in inducing this reproductive dysfunction. Research has confirmed this relationship through studies that have administered recombinant human leptin to women with hypothalamic amenorrhea, and the results suggest that leptin administration can significantly improve reproductive, thyroid, and growth hormone axes (Welt et al., 2004). The results of these studies suggest that leptin is likely a sensitive indicator of energetic availability and its availability in the body strongly mediates reproductive, neuroendocrine, and metabolic function (Nelson, 2011).

Adiponectin is a matrix-like protein that originates exclusively in adipose tissue (Maeda et al., 1996). In mice, the adiponectin homolog has been cloned as AdipoQ and ACRP30 (Scherer et al., 1995; Hu et al., 1996). In humans, two receptors for adiponectin have been cloned and are termed AdipoR1 and AdipoR2. T-cadherin may also act as a coreceptor for a signaling receptor through which adiponectin transmits metabolic signals (Meier & Gressner, 2004). Adiponectin is induced during adipocyte

differentiation and its release is stimulated by insulin (Meier & Gressner, 2004). Unlike leptin, adiponectin is negatively correlated with body mass index, with the stronger correlation existing between adiponectin levels and visceral adiposity as opposed to the protein and subcutaneous adiposity (Takahashi et al., 1996; Matsuzawa et al., 2004). Plasma adiponectin levels in humans are considered high, averaging around 5-10 μg/Ml (Matsuzawa et al., 2004). Changes in adiponectin levels are associated with a variety of health consequences. Increased serum adiponectin is associated with type-I diabetes, chronic renal failure, and anorexia nervosa. Decreased serum adiponectin is associated with type-II diabetes and coronary artery disease (Meier & Gressner, 2004).

There is a well-established negative correlation between BMI >30 kg/m^2 and adiponectin. Circulating adiponectin levels are reduced in individuals with a BMI > $30kg/m^2$ (Woodward et al., 2017). Similarly, adiponectin levels have been shown to increase in association with weight loss (Faraj et al., 2003). Plasma adiponectin concentrations are negatively correlated with BMI, body fat percentage, fasting insulin concentration, and plasma triglycerides. Moreover, adiponectin levels are positively correlated with the plasma cholesterol in HDL (Cnop et al., 2003). Caloric excess may reduce the synthesis and secretion of adiponectin. This is potentially associated with leptin deficiency or resistance, suggesting that these two metabolic hormones are both sensitive to energy balance and may play off one another (Saltiel, 2001; Meier & Gressner, 2004).

There is a clear relationship between adiponectin and glucose metabolism. High concentrations of adiponectin may be associated with a decreased risk for type II diabetes (Spranger et al., 2003). Individuals with diabetes also have lower adiponectin levels

compared to the control subjects (Matsuzawa et al., 2004). Additionally, diabetic individuals with macroangiopathy have lower adiponectin levels than those who do not have macroangiopathy (Hotta et al., 2000). Low levels of adiponectin have also been found among the Pima Indians, who have a high prevalence of diabetes and a large proportion of individuals with a BMI greater than 30 kg/m^2 (Lindsay et al., 2002). Other studies also found that adiponectin levels have a strong inverse correlation with insulin sensitivity evaluated by glucose disposal rate (Stefan et al., 2002). These studies suggest that adiponectin may have an important role in the actions of insulin and low levels of adiponectin (hypoadiponectinemia) may contribute to insulin resistance and diabetes mellitus (Matsuzawa et al., 2004). It is unclear if low levels of adiponectin are a result of genetic factors or visceral fat accumulation, however, it is possible that adiponectin exerts protective efforts on glucose metabolism (Matsuzawa et al., 2004).

Overview of Chapters

The overall goals of this dissertation work were to: 1) expand on the knowledge of human metabolic variation 2) to demonstrate the necessity for research outside of the typical research contexts and 3) to highlight key areas for future research. This work is broken into three distinct research projects each covered in the coming chapters.

The second chapter details the very first study ever conducted on leptin and adiponectin and food insecurity, as well as the first examination of these hormones among the Tsimane. Even further, it boasted the largest sample size of any study to date that looked at leptin in forager-horticulturalists and was the first to look at adiponectin in those populations. With a sample size of 148 individuals, the study explored the

relationship between the two metabolic hormones, food insecurity, and depression among the Tsimane.

The third chapter expanded on the first by increasing the sample size dramatically. With a sample of 1,670 individuals, this chapter explored the relationship between these two metabolic hormones and several key indicators of cardiovascular health, such as systolic/diastolic blood pressure, epicardial fat, liver density, hepatic steatosis, and various types of calcium build up in major vessels around the heart.

Lastly, the fourth chapter pivoted towards a methodological complication of working with market-distant populations. This chapter details a laboratory experiment where the goal was to determine whether liquid nitrogen (LN) exposure could compromise or cross-contaminate dried blood spot (DBS) samples. DBS samples are increasing in popularity among researchers working with populations such as the Tsimane, so this chapter discussed its utility and explored a potential pitfall.

In sum, these chapters are a mix of theoretically driven empirical studies, as well as methodological studies that will help create a better understanding of what we consider to be “normal” human biological variation, while also increasing the tool set required to assess variation outside of the laboratory.

CHAPTER 2

In 2020, the Global Nutrition Report stated that one in nine people is considered hungry or malnourished (WHO, 2020). Food insecurity has been a primary global health concern for decades, but research has only recently began focusing on the more nuanced complexities involved in understanding food availability as a biosocial phenomenon (Hadley & Crooks, 2012). The rise of the Covid-19 virus has exacerbated food security issues globally, with particularly harrowing impacts on mental health (Fang et al., 2021). Food insecurity can be a difficult concept to define and examine cross-culturally (Hadley & Wutich, 2009). However, the relationship between food insecurity and poor mental health has been well-documented in both Western and non-Western contexts (Coates et al., 2006; Cole & Tembo, 2011; Fang et al., 2021; Hadley & Crooks, 2012).

Depression has often been described as a by-product of modernity, however more recent research has demonstrated that depression exists even in populations that lack the risk factors found in industrialized societies (Hagen, 2003; Nesse, 2019; Stieglitz et al., 2015a). Rather, depression seems to be a response to conditions that humans have regularly faced throughout history (Nesse, 2000). Several explanations have been put forth to explore depression within the human adaptive complex. A few have suggested that there are social benefits to depression, such as increasing the ability to solve social problems through rumination (Andrews & Thomson, 2009), avoiding social costs by signaling submission (Price et al., 2004), avoiding exclusion (Allen et al., 2003), and imposing costs on partners to elicit greater investment (Hagen, 2003). Another compelling explanation argues that depression may function to conserve and reallocate energy. Termed the “host defense hypothesis”, depression may be a related to “sickness

behavior". In other words, depression may be one component of a broader adaptive response to infection or tissue injury (Trumble, et al., 2015a). One particular study in a group of forager-horticulturalists supports this explanation and found that depressive symptoms were associated with biomarkers that reflect greater immune activation. This same study also found that food insecurity was a primary predictor of depressive symptoms within this population (Stieglitz et al., 2015a).

From strictly biological and physiological perspective, food insecurity leads to undernourishment, which thereby leads to a negative energy balance in the body. Metabolic energy allocation is crucial to the shaping of human life history strategies and health outcomes. Metabolic energy is often reallocated to alternative physiological domains as a mechanism for growth, survival, and reproduction. Therefore, the human body wants to be in a state of homeostasis, where the energy being taken in is more or equal to the amount of energy being put out (Ellison, 2017). There are two metabolic hormones that play a key role in signaling metabolic energy availability in the body: leptin and adiponectin.

Leptin, also commonly known as the 'satiety hormone', is produced mostly by adipose tissue (Zhang et al., 1994). Leptin production is primarily regulated by food intake and the amount of circulating leptin is proportional to the amount of fat tissue in the body (Flier, 1998). Leptin's primary role is to convey information to the brain regarding the amount of energy stored in fat. If energy input and output are in a state of balance, then leptin directly reflects the proportion of adipose tissue in the body. But if these fat stores are being used for energy, circulating leptin levels fall drastically (Meier & Gressner, 2004). Due to its key role in appetite regulation, upon its discovery

leptin was thought to be a potential cure for excess body weight due to these metabolic functions. However, in larger bodied individuals, leptin levels are increased but the body becomes desensitized to the high levels of leptin and essentially becomes "leptin resistant". The result is that individuals with higher body fat percentages experience no decreases in appetite despite their elevated leptin levels. Because of its primary role in signaling homeostasis, leptin availability in the body is a strong mediator of reproductive, neuroendocrine, immune, cardiovascular, and metabolic function (Flier, 1998; Flier & Maratos-Flier, 2017).

Adiponectin is a matrix-like protein that originates exclusively in adipose tissue (Maeda et al., 1996). However unlike leptin, adiponectin is negatively correlated with BMI, so as body fat percentage increases, adiponectin levels actually go down (Meier & Gressner, 2004). Changes in adiponectin levels are very well associated with a variety of health consequences. For example, decreased adiponectin is associated with metabolic issues (like type-II diabetes) and cardiovascular complications (like coronary artery disease). Additionally, being in a state of positive energy balance may reduce the production of adiponectin. This is potentially associated with leptin resistance, suggesting that these two metabolic hormones are both sensitive to energy balance and may play off one another (Meier & Gressner, 2004).

Most of the studies published on leptin and adiponectin have been conducted in industrialized populations, or have utilized mouse models. When these hormones have been explored in humans, most of the research has been conducted using industrialized populations, with only a handful of notable exceptions (Bribiescas, 2001, 2005; Bribiescas & Hickey, 2006; Harries & Bribiescas, 2021).

Research in modern small-scale, subsistence populations could offer a new perspective on the relationship between depression, food insecurity, and the endocrine correlates of malnourishment. These populations tend to exhibit lifestyles and occupy environments that are much more similar to those that we evolved in and therefore research in these populations may help us to understand how these hormones operate under a more evolutionarily stable environment (Gurven & Lieberman, 2020; Trumble & Finch, 2019). Of course, it is important to note that humans evolved in a mosaic of different environments and ecologies and no modern human population can serve as a perfect proxy for human evolutionary conditions. It is at the very least clear that industrialized populations occupy a very novel environment that likely obscures what may be the true extent of human biological variation. This study seeks to examine 1) if there is a relationship between food security and these key metabolic hormones, and 2) explore if there is any hormonal association with depressive symptoms.

Methods

Study Population

The Tsimane (population ~17,000) occupy over 90+ villages in the lowlands of Bolivia (Gurven et al., 2017). Most of their diet comes from horticulture (66% of calories), hunting (17%), fishing (7%), and fruits and nuts gathered from the forest (6%) (Kraft et al., 2018). The Tsimane have relatively high fertility (total fertility rate = 9 births per woman), high physical work load (~5–6 h/day spent in lifestyle-moderate activity), and minimal access to modern healthcare, sanitation and electricity (Dinkel et al., 2020; Gurven et al., 2017). The Tsimane Health and Life History Project (THLHP) has been working with the Tsimane for the last two decades.

The sample consisted of 148 Tsimane individuals, ranging in age from 36 to 92 years (mean age = 58 years). Of the individuals in the sample, 49% were female, the average body mass index (BMI) was 23.86, and 34% identified as food insecure.

Table 1

Sample Characteristics-median and range for study variables by sex and population.

	Mean	**Standard Deviation**	**Range**
Age	58	10.73	36-62
Leptin (pg/mL)	2.44	3.22	0.05-24.21
Adiponectin (pg/mL)	7.97	4.79	0.16-20.67
Body Fat (%)	22.28	7.75	5.80-41.30
BMI (kg/m^2)	23.86	3.13	15.74-33.15
	Proportion	**Standard Error**	**95% Confidence Intervals**
Food Insecure (%)	34	5.66	23.79-47.02
Male (%)	50.99	5.81	40.24-63.74
Depression Score (%)	35.8	6.54	23-50

Depression Survey and Food Insecurity

Food insecurity was evaluated as part of depression inventory based on validated depression scales used among diverse populations, including Beck's Depression Inventory, the Hamilton Depression Rating Scale, and the Center for Epidemiologic Studies Depression Scale (Stieglitz et al., 2014; Stieglitz et al., 2015; Stieglitz et al., 2015a). An 18-item interview was prepared utilizing most of the symptoms contained in the previous scales (Stieglitz et al., 2015b). Responses to each symptom were recorded on a scale as rarely (1), occasionally (2), often (3), and always (4). The symptoms contained emotional, somatic, and cognitive components. The survey was translated from Spanish

into Tsimane by two bilingual Tsimane anthropologists. With translation accuracy tested by back-translating the survey into Spanish by a different Tsimane anthropologist. Discussions among the three Tsimane ensued until a translation was found that captured the essence of each item. To minimize recall bias, participants were queried about prevalence of symptoms over the past month. One of the questions asked participants if they felt they had insufficient food (yes or no); those that replied in the affirmative were designated to be food insecure (Stieglitz et al., 2014; Stieglitz et al., 2015; Stieglitz et al., 2015a).

Interviews were conducted in a private location in the Tsimane language by a Tsimane researcher with multiple years of relevant experience. Adults were recruited regardless of their health status. Older adults are over-represented given the THLHP's focus on aging. All methods were approved by Institutional Review Boards at University of New Mexico and University of California Santa Barbara, and by the Tsimane government, village leaders and study participants (Stieglitz et al., 2015).

Anthropometric and Biomarker Collection

Weight and bodyfat measurements were collected with a Tanita BC-1500 scale, and height with a SECA 213 portable stadiometer. Fasting morning blood draws were conducted as a part of routine medical surveillance. A vacutainer of blood without anticoagulant was allowed to clot, and then serum was separated via centrifugation (1500g for 10 minutes) and frozen in liquid nitrogen. Specimens were transported on dry ice to the Arizona State University (ASU) Evolutionary Medicine and Biodemography laboratory and stored at -80C for up to four years before analyses.

Laboratory Methods

Leptin and adiponectin were measured by the author at the ASU Evolutionary Medicine and Biodemography laboratory. Leptin levels were analyzed using DRG Leptin Sandwich ELISA (EIA-2395R). The DRG Leptin ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle following manufacturers recommendations. Adiponectin levels were analyzed using AssayPro Human Adiponectin ELISA (EA2500-1), following manufacturers recommendations.

Statistical Methods

Leptin and adiponectin were log transformed for normality prior to analysis. Linear regression models examined the associations between leptin and adiponectin and food insecurity, controlling for bodyfat, age, sex, and BMI status (BMI >30kg/m^2). Model selection was assessed via Akaike's Information Criteria (AIC), and all models were checked to ensure variance inflation factors were low, indicating no co-linearity.

Ethics Statement

Informed consent was collected at the level of the participant, the community, and the Tsimane governing body (*Tsimane Gran Consejo*), and the procedures were approved by the institutional review boards at the University of California, Santa Barbara (protocol number 28-21-0788), and University of New Mexico (study ID 07-157).

Results

Food Insecurity and Metabolic Hormones

Individuals suffering from food insecurity had significantly lower log leptin levels (b=-0.414, p=0.009, CI -0.108 to -0.720), controlling for body fat percentage, age, sex, and BMI status, see Table 3. Higher body fat and BMI status were both associated with

higher levels of leptin (all p<0.006), while age and male sex were both associated with lower levels of leptin (all p <0.021), Table 3, figure 1. There were no associations between food insecurity and log adiponectin levels (0.408), Table 1.

Table 2

Associations between log adiponectin and food insecurity

	Beta	p-value	95% Confidence Interval
Food Insecure	-0.09	0.408	[-0.321, 0.132]
Body Fat (%)	0.002	0.776	[0.014, 0.019]
Age	0.02	0.000	[0.012, 0.032]
Sex	0.06	0.621	[-0.189, 0.315]
BMI>30	0.10	0.746	[-0.519, 0.721]
Constant	0.60	0.088	[-0.090, 1.284]

r2= 0.196.

Table 3

Associations between log leptin and food insecurity

	Beta	p-value	95% Confidence Interval
Food Insecure	-0.41	0.009	[-0.720, -0.108]
Body Fat (%)	0.07	0.000	[0.046, 0.088]
Age	-0.02	0.021	[-0.031, -0.002]
Sex	-0.59	0.001	[-0.926, -0.261]
BMI>30	1.17	0.006	[0.348, 1.996]
Constant	0.19	0.706	[-0.800, 1.176]

r2=0.6136.

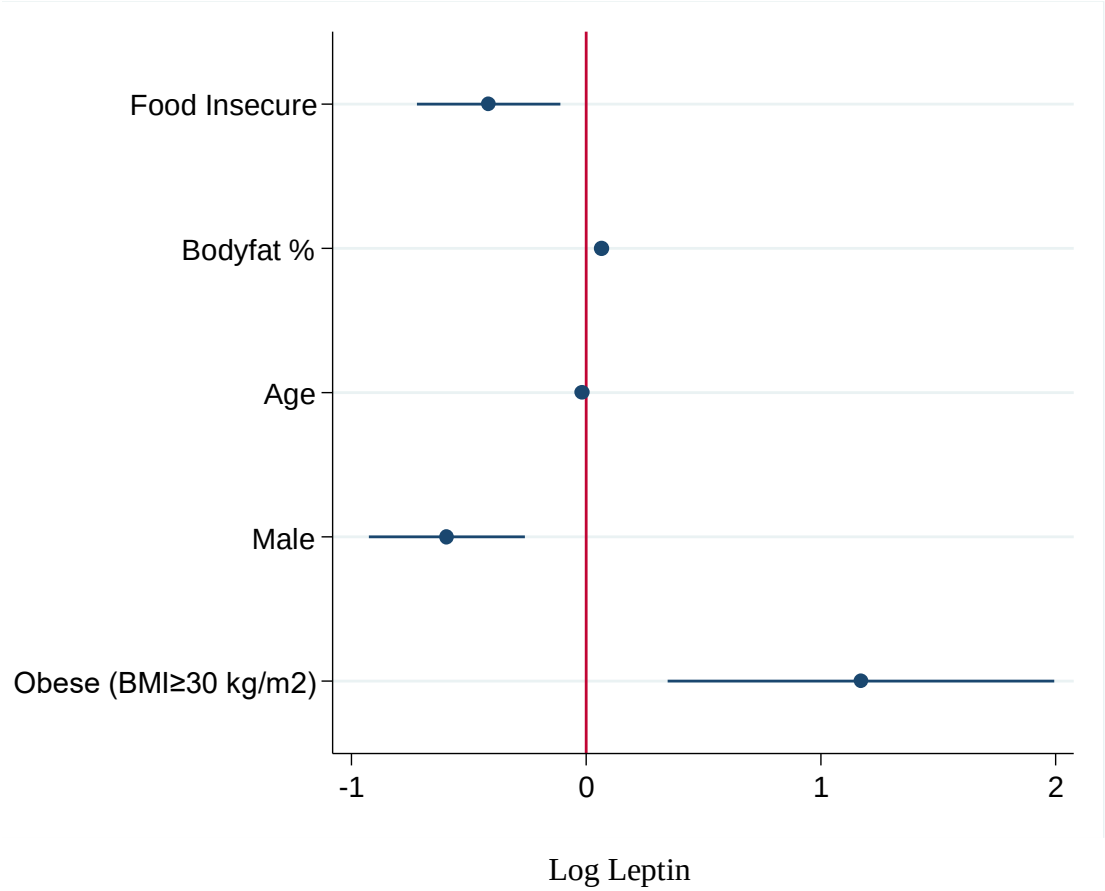


Figure 1. Associations between log leptin and food insecurity

Depression and Metabolic Hormones

Higher log adiponectin was associated with lower depression scores (b=-3.453, p=0.004), controlling for age, sex and body fat. In this model, age positively predicted depression score (b=0.183, p=0.003), while body fat (b=-0.202 , p=0.014), and male sex (b=-6.552, p<0.001) were associated with lower depression scores, see Table 4 and Figure 2. There was no association between leptin and depression score (p=0.598).

Table 4

Associations between depression and adiponectin

	Coefficient	p-value	Standard Error	95% Confidence Interval
Log Adiponectin	-3.43	0.004	1.15	[-5.716, -1.150]
Body Fat (%)	-0.16	0.059	0.08	[-0.327, 0.006]
Age	0.17	0.005	0.06	[0.053, 0.288]
Sex	-6.35	0.000	1.29	[-8.903, -3.790]
BMI>30	-6.03	0.082	3.43	[-12.840, 0.773]
Constant	39.44	0.000	3.72	[32.064,46.810]

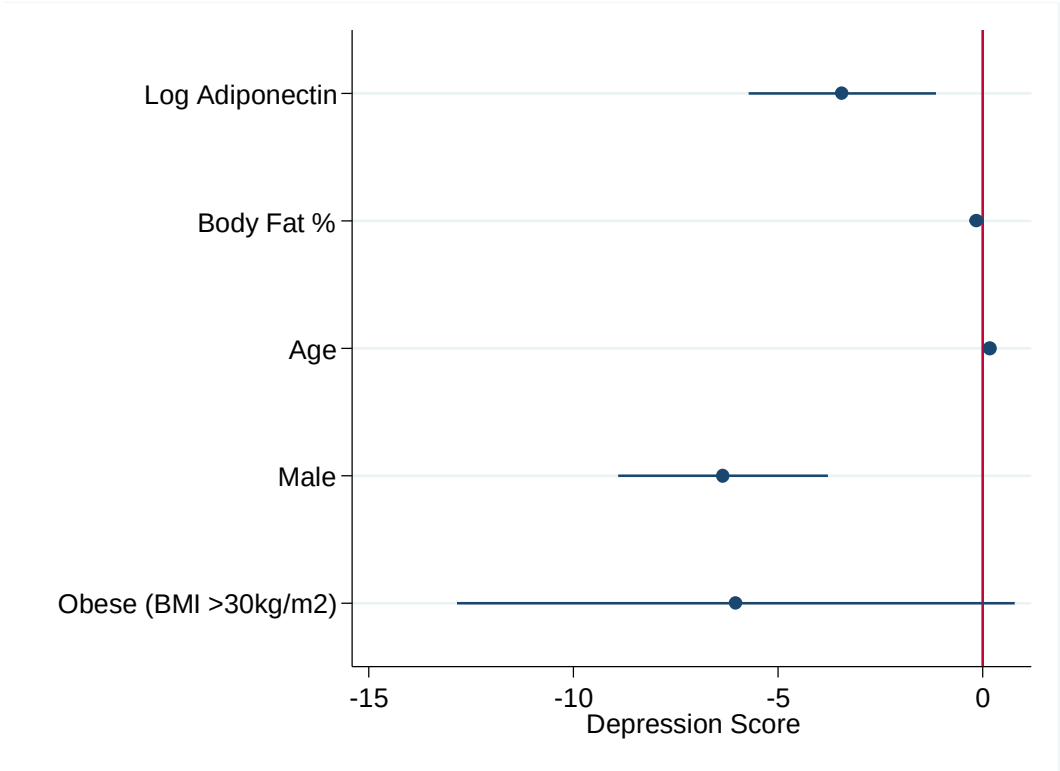


Figure 2. Associations between depression score and log adiponectin

Discussion

The relationship between leptin levels and major depressive disorder has been extensively studied in urban, industrialized populations. Low leptin levels have been found to be associated with depression in humans and other mammals. Additionally, obese individuals are 20% more likely to exhibit symptoms of depression and because high BMI is related to leptin resistance, it has been suggested that leptin resistance may also be associated with depressive symptoms (Lu, 2007). Though it should be noted that depression among obese individuals could also be related to weight bias experienced in many industrialized societies (Brewis et al., 2018). Along similar lines, studies have also demonstrated an association between adiponectin and depressive symptoms. Lower plasma adiponectin concentration was found in patients with major depressive disorder (Leo et al., 2006). The results of the present study support a potential relationship between lower adiponectin concentration and depression, suggesting that this relationship is not merely a function of industrialization, but rather a deeply engrained physiological relationship.

The relationship between food availability and metabolic hormones is not well researched. Since leptin and adiponectin are key indicators of energy availability in the body, changes in these hormones is expected in contexts where adequate nutrition is limited. However, little research has been done to directly explore the specifics of the relationship between food availability and leptin or adiponectin. One study explored the association between leptin levels and mistreated children and found that leptin levels were lowered in children who experienced mistreatment. Mistreated children often experience food insecurity, but in this study no associations were made directly between

food insecurity and leptin levels (Danese et al., 2014). Additionally, it has also been posited that there is an adaptive relationship between leptin and fertility in women in more food insecure environments, but again this direct link has not been tested (Power & Schulkin, 2008). This present study suggests that there is a relationship between food insecurity and low leptin levels, and thus this relationship should be explored further.

The results of this study support the idea that depression may be related to the energy conservation and reallocation experienced during periods of immune activation (Stieglitz et al., 2015b). Leptin and adiponectin are both key indicators of energetic availability. Therefore, it is probable that there is a strong relationship between increased immune activation, the resulting destabilized energetic homeostasis and increased risk of depression. Future studies should explore the association between these metabolic hormones and immune biomarkers to further explore this relationship.

Previous studies of the association between leptin and adiposity in traditional subsistence populations have demonstrated the problem of using industrialized populations as models for all of human variation. Studies conducted among the Ache Amerindians of Eastern Paraguay demonstrated that ecological conditions and activity levels can significantly impact the strong relationship between body fat percentage and leptin levels that are observed in the American population (Bribiescas, 2001, 2005; Bribiescas & Hickey, 2006). For example, Ache men have lower leptin concentrations when compared to American distance runners, despite greater adiposity (Bribiescas & Hickey, 2006). These studies have demonstrated the importance of researching physiological variation outside of industrialized contexts, as these contexts may represent only one piece of the spectrum and is thus not representative of the whole of human

variation (Bribiescas & Hickey, 2006). That being said, this present study found a strong positive association between body fat percentage and leptin concentrations, suggesting that among the Tsimane the relationship between these two variables is more in line with the trends observed in industrialized populations.

Limitations

While the studies on the Ache were foundational, they were fairly limited by small sample sizes (highest n=33). Along similar lines, the present study is also limited by a relatively small sample size (n=148). However, this study's sample size is still significantly larger than all previous studies of leptin in non-industrialized populations combined. Additionally, this was the first study that explored adiponectin levels in a non-industrialized population.

Another limitation of this study is that food insecurity was evaluated based on a singular question from a depression survey. A full food insecurity questionnaire by another research town found a similar proportion of Tsimane individuals reported food insecurity (Bethancourt et al., 2021). Importantly, this is the first study to date to show that leptin was associated with food insecurity, even in a relatively lean population.

Conclusion

This study found significant relationships between 1) leptin and food insecurity and 2) adiponectin and depression scores. The results of this study lend support to the hypothesis that depression is a strategy that serves to regulate energy allocation, especially when faced with sickness in food-limited environments because the hormones leptin and adiponectin play a crucial role in metabolic function and energy availability.

Therefore, leptin and adiponectin are potentially important physiological mediators of depression's effect on nutritional status. Additionally, this study demonstrated the importance of researching global physiological variation outside of industrialized contexts. This study was 1) the largest study to date on leptin levels in a small, subsistence population and 2) the first study to date on adiponectin levels in a small, subsistence population. Other studies have demonstrated that industrialized populations are not ideal references for the whole of human variation, and thus research should continue to explore human variation outside of these contexts.

CHAPTER 3

An estimated 32% of all deaths worldwide are attributed to cardiovascular diseases (CVDs), which encompass a suite of disorders that affect the heart and blood vessels including cardiovascular disease, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease, deep vein thrombosis, and pulmonary embolism. CVD is the largest killer worldwide, with an estimated 17.9 million fatalities in 2016 (WHO, 2017). There are a number of behavioral risk factors involved with the development of CVDs, such as a high-calorie/high-fat diet, physical inactivity, and tobacco or alcohol use (WHO, 2021). It is no surprise that CVD is one of the top global health concerns.

However, the current understanding of CVD and its associated risks has been limited in its scope. Much of the research of CVD (and many other health-related issues) has been focused on primarily rich, industrialized populations occupying calorie rich environments and sedentary lifestyles (Pontzer et al., 2018). The assumption that these societies are representative of the extent of human variation is problematic and many studies have demonstrated that variation in ecology, subsistence, population history, culture, parasite/pathogen loads, and discrimination can influence human physiology (Ellison, 2017; Gurven & Lieberman, 2020; Konner & Worthman, 1980; Kaplan et al., 2017; Trumble & Finch, 2019. Instead of attempting to determine what "normal" human physiology is, researchers should instead focus on the extent of human variation and what variables influence such variation (Trumble and Schneider-Crease 2020).

This research project seeks to explore the relationship between CVDs and two key metabolic hormones: leptin and adiponectin. High leptin levels and low adiponectin

levels are known to be associated with CVD in rich, industrialized contexts, but this relationship has been unexplored in relatively smaller populations that are distant from markets, engage in high levels of physical activity, and consume a low-fat, low-calorie diet. Therefore, this project explores the relationship between CVD and metabolic hormones among the Tsimane forager-horticulturalists of lowland Bolivia and their sister population, the Moseten.

Background

The Tsimane and Moseten

The Tsimane are an indigenous forager-horticulturalist group occupying the tropical lowlands of Bolivia. They are considered one of the most isolated indigenous groups living in Bolivia. The Tsimane primarily subsist on slash-and-burn horticulture, fishing, hunting, and seasonal gathering (Gurven et al 2017). They produce mainly plantains, rice, corn, and sweet manioc. They hunt a wide variety of neotropical mammals and fish in the local rivers, lagoons, and streams. They seasonally gather fruits and other foods, such as nuts and honey. The Tsimane occupy about 90 villages, each ranging from 50 to 500 individuals. These villages are settled along the Maniqui, Quiquibey, and Mato rivers. Their current overall population size is estimated to be about 17,000 Tsimane, with a population growth rate of over 3.5% (Gurven et al., 2017). The Moseten are a sister population to the Tsimane, who have a more market-based lifestyle. The Moseten have more access to schooling, clean water and electricity, and many are monolingual Spanish speakers and no longer speak the Moseten language. Moseten engage in more cash cropping than Tsimane. The Moseten show higher rates of BMI>30 kg/m^2 and type 2 diabetes and have more processed foods in their diet (Kraft et al 2018).

The Tsimane have been identified as a population with remarkable cardiovascular health. The Tsimane have the lowest reported levels of coronary artery disease of any population recorded to date (Kaplan et al., 2017), as well as low levels of hypertension (Gurven et al., 2012), and atrial fibrillation (Rowan et al., 2021). The Tsimane's diet and high activity lifestyle are often credited with the low prevalence of coronary artery disease risk factors, such as coronary artery calcium (CAC) levels, cholesterol levels, and blood lipid levels, as well as potential immune relate factors (Gurven et al., 2016). Among the Tsimane there are low levels of several key CVD risk factors, including a low prevalence of hypertension, low lipid and glucose concentrations, and low rates of smoking. Furthermore, unlike in many urban populations, there is little evidence of age-related increases in hypertension, diabetes, excess weight, or hypercholesterolemia (Kaplan et al., 2017).

Prevalence of coronary artery atherosclerosis was measured using CAC scoring, a non-invasive and highly sensitive imaging technique (Kaplan et al 2017). In a sample of 705 Tsimane over age fifty, 85% of the Tsimane had no CAC, while only 15% of an age-matched comparison sample from the US lacked CAC. In the US, the median CAC score for a male aged 70+ is over 400 Agatson units, while only one Tsimane had a calcium score over 400. These low levels of CAC extend into older age individuals. Tsimane men have higher CAC scores than Tsimane women, but Tsimane men still had lower CAC scores in comparison to Japanese women who were previously considered to have the lowest CAC scores for any population. Factors associated with CAC scores of greater than 0 in the Tsimane were age, body-fat percentage, hs-CRP levels, and erythrocyte

sedimentation rate. In contrast, younger age, female sex, and lower triglyceride levels were more greatly associated with CAC absence (Kaplan et al., 2017).

The mechanisms that protect the Tsimane from CAC development remain unclear (Gurven et al., 2016). There are several potential explanations including their subsistence lifestyle (diet and energy expenditure), genetics, inflammation, and immune regulation (Gurven et al., 2016, Raichlen et al., 2016). The low LDL or a low LDL-to-HDL ratio seen in the Tsimane may potentially have atheroprotective effects which may be further augmented by the Tsimane lifestyle. Though genetics do play a minor role in the development of coronary artery disease, the extent of its genetic effects among the Tsimane remains unclear. However, the increasing cholesterol levels seen in recent years among the Tsimane suggest that they are not likely genetically protected against heart disease (Kaplan et al., 2017). While there is some evidence that atherosclerosis is an inflammatory disease, a high inflammatory burden has been observed with a low prevalence of CAC for all inflammatory markers in the Tsimane. This suggests that in populations with high pathogen burdens and chronic inflammation, atherosclerosis may not be associated with inflammation, particularly when combined with low LDL concentrations (Gurven et al., 2009; 2016; Kaplan et al., 2017).

Leptin

Leptin is a 16KDa protein of 167 amino acids and is a product of the ob gene located on chromosome 7 in humans. Leptin, also commonly known as the ‘satiety hormone’, is synthesized and secreted mostly by white adipose tissue (Zhang et al., 1994). However, leptin can also be produced by the heart, vascular smooth muscle, placental tissue, digestive epithelia, the pancreas, and the lungs (Hou & Lou, 2011; Malli

et al, 2010; Larsson & Ahren, 1996). Leptin production is primarily regulated by food intake and the amount of circulating leptin is proportional to the amount of fat tissue in the body (Flier, 1998). Leptin conveys information to the hypothalamus regarding the amount of energy stored in fat (Meier & Gressner, 2004). When fat stores are being used for energy, circulating leptin levels fall drastically. This may suggest that leptin functions as a starvation signal that stimulates food intake when levels begin to fall, rather than a satiety signal that discourages food intake when leptin levels rise (Flier, 1998; Flier & Maratos-Flier, 2017).

Sexual dimorphism in leptin levels exists in humans. Leptin levels decline in association with rising testosterone in adolescent males, likely due to the energetic costs of a high testosterone phenotype (Jasienska et al., 2017). In contrast, leptin levels rise in association with estrogen both during and after puberty, likely related to changes in body composition that occur during the pubertal transition (Garcia-Mayor et al., 1997). Adult leptin levels are associated with body fat percentage and age. Females exhibit declines in leptin concentrations following the menopausal transition (Rosenbaum et al., 1996), though postmenopausal females still have higher leptin levels than age-matched males, even when controlling for body fat (Saad et al., 1997).

Leptin levels are sensitive to a variety of factors. For example, glucocorticoids and insulin are thought to promote leptin production (Widjaja et al, 1998). Leptin concentrations may also rise in association with sepsis or infection (La Cava et al., 2004). However, acute activation of the inflammatory system or chronic inflammation can lead to a reduction in leptin levels (Popa, 2005). Leptin is also thought to increase sympathetic nervous system activity, contribute to neo-vascularization processes, mediate wound

healing, assist in bone turnover or skeletal development, and may even mediate fertility (Cao et al, 1997; Bouloumie et al, 1998; Frank et al, 2000; Gordeladze & Reseland, 2003; Keisler et al, 2019).

Leptin plays a strong role in cardiovascular diseases (CVD) presenting in individuals with a BMI of greater than 30 kg/m^2. In these individuals, serum leptin levels are elevated and correlate with body mass index. A BMI of greater than 30 kg/m^2 is associated with high risk of developing CVD symptoms, such as hypertension, coronary atherosclerosis, and myocardial hypertrophy (Hou & Luo, 2011). Selective leptin resistance is also observed in these individuals, disrupting the hypothalamus and its control of homeostasis, leading to an imbalance between food intake and energy expenditure. This results in lipid deposition in the heart and blood vessels leading to compromised cardiovascular function (Wilsey & Scarpace, 2004).

Several studies have demonstrated strong, positive associations between elevated leptin levels and cardiovascular complications. For example, high leptin levels are associated with myocardial infarction (Wallerstedt et al., 2004), hypertension (Wallerstedt et al., 2004; Sierra-Johnson et al., 2007), diabetes (Welsh et al., 2009), and coronary heart disease and stroke (Sierra-Johnson et al., 2007; Liu et al., 2010). Interestingly, individuals with a BMI>30 kg/m^2 with leptin deficiency are overweight, but hypotensive (Rahmouni et al., 2005). This suggests that leptin's role in blood pressure regulation is more pronounced than the role of body weight alone. Leptin may also play a role in atherosclerosis. Some clinical studies have shown that plasma leptin concentrations are associated with atherosclerosis and leptin receptors have been found in human atherosclerosis (Reilly et al., 2004; McMahon et al., 2011). Other studies suggest

that elevated plasma leptin levels are associated with cardiac hypertrophy and remodeling (Hou & Lou, 2011; Madani et al., 2006). Additionally, leptin levels may be inversely correlated with micro vascular flow rate (Tigno et al., 2003). Leptin is clearly a pleiotropic hormone that plays a role in many biological tissues.

Adiponectin

Adiponectin is a matrix-like protein that originates exclusively in adipose tissue (Maeda et al., 1996). In mice, the adiponectin homolog has been cloned as AdipoQ and ACRP30 (Scherer et al., 1995; Hu et al., 1996). In humans, two receptors for adiponectin have been cloned and are termed AdipoR1 and AdipoR2. T-cadherin may also act as a coreceptor for a signaling receptor through which adiponectin transmits metabolic signals. Adiponectin is induced during adipocyte differentiation and its release is stimulated by insulin (Meier & Gressner, 2004). Unlike leptin, adiponectin is negatively correlated with body mass index, with the stronger correlation existing between adiponectin levels and visceral adiposity as opposed to the protein and subcutaneous adiposity (Takahashi et al., 1996; Matsuzawa et al., 2004). Plasma adiponectin levels in humans are considered high, averaging around 5-10 μg/Ml (Matsuzawa et al., 2004). Changes in adiponectin levels are associated with a variety of health consequences. Increased serum adiponectin is associated with type-I diabetes, chronic renal failure, and anorexia nervosa. Decreased serum adiponectin is associated with type-II diabetes and coronary artery disease (Meier & Gressner, 2004).

Adiponectin is thought to have several beneficial effects on cardiovascular function because of its anti-inflammatory, anti-apoptic, antioxidant, and vasorelaxant properties (Kadowaki & Yamauchi, 2005). It is suggested that low adiponectin levels

may be associated with cardiovascular complications in individuals with high BMI (Hung et al., 2008). Additionally, some studies have found low levels of plasma adiponectin among individuals with hypertension (Mallamaci et al., 2002). Furthermore, low levels of adiponectin have been found to be associated with ischemic heart disease (Kumada et al., 2003). Some evidence suggests that adiponectin suppresses the development of atherosclerosis and liver fibrosis (Meier & Gressner, 2004). Therefore, hypoadiponectinemia might be an important risk factor for atherosclerotic disease (Kumada et al., 2003).

However, the literature often discusses a purported "adiponectin paradox", because on the other hand, high leptin levels are associated with advanced cardiovascular disease (Berg & Scherer, 2005). Therefore, adiponectin is often used as a clinical biomarker for cardiovascular disease (Woodward et al., 2017). This relationship between adiponectin, its cardio-protective effects, and cardiovascular disease is complex, and the current state of the literature needs to be expanded.

Methods

Sample Characteristics

The sample consisted of 1670 Tsimane and Moseten individuals, ranging in age from 32 to 94 years (mean age = 60.44 years). Of the individuals in the sample, 51.2% were female, 7.8% have a BMI >30 kg/m^2, the average body mass index (BMI) was 24.17 kg/m^2.

Anthropometric and Biomarker Collection

Weight and body fat measurements were collected with a Tanita BC-1500 scale, and height with a SECA 213 portable stadiometer. Fasting morning blood draws were conducted as a part of routine medical surveillance. A vacutainer of blood without anticoagulant was allowed to clot, and then serum was separated via centrifugation (1500g for 10 minutes) and frozen in liquid nitrogen. Specimens were transported on dry ice to the Arizona State University (ASU) Evolutionary Medicine and Biodemography laboratory, and stored at -80C for up to four years before analyses. Coronary Artery Calcium, Epicardial fat, and liver density, and aortic valve and thoracic aortic calcium were measured with an electrocardiogram (ECG)-gated CT imaging using a 16-detector row scanner (GE Brightspeed, Milwaukee, WI, USA). A licensed radiological technician acquired a single, ECG-gated scan under the supervision of a team cardiologists. A core laboratory performed calcium scoring of the coronaries using semi-automatic software (GE SmartScore 4.0, Milwaukee, WI, USA).

Several cardiovascular measures were chosen as outcome variables because they are all heavily associated with metabolic status. Coronary artery calcium (CAC) refers to the amount of calcium that has built up in the coronary arteries. High levels of CAC are associated with a markedly increased risk of coronary heart disease (Greenland, 2004). Epicardial fat is a visceral fat deposit located around the heart. Excess epicardial fat is associated with coronary atherosclerosis, hypertension, and many metabolic disorders (Bertaso et al., 2013). Low liver density and hepatic steatosis (fatty liver disease) are also found to be associated with an increased risk of cardiovascular complications (Ismaiel &

Dumitrascu, 2019). Aortic valve calcium refers to the amount of calcium that has built up on the aortic valve in the heart, which ultimately would restrict blood flow into the heart (Mohler, 2004). Thoracic aortic calcification (TAC) refers to the amount of calcium present in the aorta in the thoracic cavity and is associated with an increased risk of cardiovascular disease (Desai et al., 2018).

Laboratory Methods

Leptin and adiponectin were measured by the author at the ASU Evolutionary Medicine and Biodemography laboratory. Leptin levels were analyzed using DRG Leptin Sandwich ELISA (EIA-2395R). The DRG Leptin ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle following manufacturers' recommendations. Adiponectin levels were analyzed using AssayPro Human Adiponectin ELISA (EA2500-1), following manufacturers' recommendations.

Statistical Methods

Leptin and adiponectin were log transformed for normality prior to analysis. Linear regression models examined the associations between leptin and adiponectin and several CVD variables (systolic and diastolic blood pressure, CAC, log epicardial fat, and liver density). Logistic regression models examined the associations between leptin and adiponectin and hepatic steatosis and CAC>=1 and CAC>100. Zero-inflated negative binomial models explored the associations between leptin and adiponectin and CAC, thoracic calcium, and aortic valve calcium. All models controlled for bodyfat, age, sex and population (Tsimane vs Moseten). Liver and CAC models also include C-Reactive Protein.

Ethics Statement

Informed consent was collected at the level of the participant, the community, and the Tsimane governing body (*Tsimane Gran Consejo*), and the procedures were approved by the institutional review boards at the University of California, Santa Barbara (protocol number 28-21-0788), and University of New Mexico (study ID 07-157).

Results

Leptin and CVD

Leptin was found to be positively associated with systolic blood pressure (b=2.39, p=0.000, 95% CI= 1.31, 3.48) and hepatic steatosis (b=1.04, p=0.036, 95% CI= 0.07, 2.00), see Tables 5 & 9. Leptin was not associated with the odds of having any thoracic calcium but was positively associated with the extent of any thoracic calcium (p=0.001, 95% CI= 1.08, 1.32), see Table 13. Leptin was found to be negatively associated with liver density (b= -2.1, p=0.000, 95% CI= -2.71, -1.55), see Table 8. Additionally, low leptin levels were associated with the odds of having no aortic valve calcium (p=0.018, 95% CI= -0.50, -0.05), see Table 14.

Adiponectin and CVD

Adiponectin closely approached positive significance with diastolic blood pressure (b=0.65, p=0.060, 95% CI= -0.03, 1.33), see Table 6. Adiponectin was not associated with the odds of having aortic valve calcium but was positively associated with the extent of any aortic valve calcium (p<0.001, 95% CI= 1.36, 2.32), see Table 14.

Table 5

Associations between systolic BP, log leptin and log adiponectin

	Coefficient	Standard Error	t score	p-value	95% Confidence Interval
Tsimane	-3.79	1.13	-3.36	0.001	-6.00, -1.58
Male	3.76	1.14	3.30	0.001	1.53, 6.00
Age	0.39	0.05	8.27	0.000	0.30, 0.49
Body Fat (%)	0.19	0.07	2.86	0.004	0.06, 0.32
Adiponectin	0.58	0.54	1.09	0.278	-0.47, 1.64
Leptin	2.39	0.55	4.34	0.000	1.31, 3.48
Constant	89.44	3.28	27.30	0.000	83.01, 95.87

Table 6

Associations between diastolic BP, log leptin and log adiponectin

	Coefficient	Standard Error	t score	p-value	95% Confidence Interval
Tsimane	-5.13	0.72	-7.09	0.000	-6.55, -3.71
Male	3.56	0.73	4.88	0.000	2.13, 4.99
Age	0.16	0.03	5.16	0.000	0.10, 0.22
Body Fat (%)	0.22	0.04	5.09	0.000	0.13, 0.30
Adiponectin	0.65	0.34	1.88	0.060	-0.03, 1.33
Leptin	0.53	0.35	1.51	0.131	-0.16, 1.23
Constant	61.41	2.11	29.13	0.000	57.27, 65.55

Non-Significant Findings

Neither leptin nor adiponectin were found to be associated with log epicardial fat, see Table 7. Additionally, neither was associated with the presence or extent of CAC, CAC>=1, or CAC>100, see Tables 10-12. Adiponectin was not associated with systolic

blood pressure (Table 5), and leptin was not associated with diastolic blood pressure (Table 6). Adiponectin was not associated with liver density, hepatic steatosis, or the presence/extent of thoracic calcium (Tables 8, 9, & 13).

Table 7

Associations between log epicardial fat, log leptin and log adiponectin

	Coefficient	**Standard Error**	**t score**	**p-value**	**95% Confidence Interval**
Tsimane	-0.15	0.03	-4.68	0.000	-0.22, -0.89
Male	-0.17	0.03	-5.20	0.000	-0.24, -0.11
Age	0.02	0.00	10.98	0.000	0.01, 0.02
Body Fat (%)	0.01	.00	7.21	0.000	0.01, 0.02
Adiponectin	0.02	0.02	1.19	0.234	-0.01, 0.05
Leptin	0.02	0.02	1.14	0.253	-0.01, 0.05
Constant	-0.37	0.09	-3.91	0.000	-0.56, -0.19

Table 8

Associations between liver density, log leptin and log adiponectin

	Coefficient	**Standard Error**	**t score**	**p-value**	**95% Confidence Interval**
Tsimane	4.44	0.89	4.97	0.000	2.69, 6.20
Male	-2.00	0.64	-3.13	0.002	-3.26, -0.75
Age	-0.04	0.03	-1.56	0.118	-0.10, 0.01
Log CRP	-0.95	0.29	-3.29	0.001	-1.52, -0.38
Adiponectin	0.42	0.30	1.39	0.166	-0.17, 1.01
Leptin	-2.13	0.30	-7.20	0.000	-2.71, -1.55
Constant	70.02	1.90	36.81	0.000	66.29, 73.75

Table 9

Associations between hepatic steatosis, log leptin and log adiponectin

	Odds Ratio	**Standard Error**	**z score**	**p-value**	**95% Confidence Interval**
Tsimane	0.06	0.06	-3.08	0.002	0.01, 0.36
Male	3.64	4.42	1.06	0.289	0.34, 39.46
Age	2.02	0.05	0.29	0.770	0.92, 1.11
Log CRP	1.71	0.99	0.93	0.352	0.55, 5.29
Adiponectin	0.71	0.27	-0.89	0.375	0.34, 1.50
Leptin	2.82	1.39	2.10	0.036	1.07, 7.41
Constant	0.00	0.01	-2.04	0.041	7.72, 0.79

Table 10

Associations between CAC, log leptin and log adiponectin

CAC	**IRR**	**Standard Error**	**z score**	**p value**	**95% Confidence Interval**
Age	1.03	0.02	1.49	0.135	0.99, 1.06
Tsimane	0.34	0.18	-2.01	0.044	0.12, 0.97
Adiponectin	1.07	0.20	0.39	0.695	0.75, 1.54
Leptin	0.06	0.15	-0.29	0.775	0.70, 1.31
Log CRP	1.48	0.23	2.49	0.013	1.09, 2.01
Constant	13.29	15.75	2.18	0.029	1.30, 135.58
Inflate					
Male	-1.08	0.23	-4.77	0.000	-1.52, -0.63
Age	-0.07	0.01	-5.64	0.000	-0.10, 0.05
Constant	6.23	0.81	7.68	0.000	4.64, 7.81

Table 11

Associations between CAC>=1, log leptin and log adiponectin

	Odds Ratio	Standard Error	z score	p-value	95% Confidence Interval
Tsimane	3.11	0.69	5.11	0.000	2.01, 4.81
Male	1.07	0.01	7.00	0.000	1.05, 1.09
Log CRP	1.13	0.12	1.19	0.233	0.92, 1.39
Adiponectin	0.83	0.09	-1.78	0.075	0.68, 1.02
Leptin	1.16	0.12	1.44	0.150	0.95, 1.43
Constant	0.00	0.00	-9.27	0.000	0.00, 0.01

Table 12

Associations between CAC>=100, log leptin and log adiponectin

	Odds Ratio	Standard Error	z score	p-value	95% Confidence Interval
Age	1.08	0.02	4.67	0.000	1.05, 1.12
Male	4.18	1.71	3.50	0.000	1.88, 9.31
Log CRP	1.75	0.39	2.53	0.011	1.13, 2.70
Adiponectin	0.76	0.13	-1.54	0.125	0.54, 1.08
Leptin	1.36	0.25	1.68	0.093	0.95, 1.94
Constant	0.00	0.00	-7.13	0.000	6.69, 0.00

Table 13

Associations between TAC, log leptin and log adiponectin

CAC	IRR	Standard Error	z score	p value	95% Confidence Interval
Age	1.08	0.01	11.40	0.000	1.07, 1.09
Tsimane	0.53	0.10	-3.26	0.001	0.36, 0.78
Adiponectin	0.98	0.06	-0.38	0.702	0.88, 1.09
Leptin	1.19	0.06	3.43	0.001	1.08, 1.32
Log CRP	1.18	0.08	2.55	0.011	1.03, 1.34
Constant	4.81	2.29	3.30	0.001	1.89, 12.22
Inflate					
Male	-0.40	0.23	-1.69	0.092	-0.86, 0.06
Age	-0.17	0.02	-8.81	0.000	-0.21, -0.13
Constant	8.70	1.07	8.16	0.000	6.61, 10.79

Table 14

Associations between aortic valve calcium, log leptin and log adiponectin

CAC	IRR	Standard Error	z score	p value	95% Confidence Interval
Age	1.04	0.01	4.36	0.000	1.02, 1.06
Tsimane	1.33	0.31	1.25	0.212	0.85, 2.10
Male	3.24	0.75	5.06	0.000	2.05, 5.10
Body Fat %	1.04	0.02	2.20	0.028	1.00, 1.07
Adiponectin	1.78	0.24	4.23	0.000	1.36, 2.32
Constant	0.58	0.43	-0.73	0.466	0.14, 2.48
Inflate					
Male	-0.86	0.25	-3.49	0.000	-1.34, -0.38
Age	-0.11	0.01	-7.89	0.000	-0.14, -0.09
Body Fat %	0.04	0.01	2.66	0.008	0.01, 0.06
Leptin	-0.27	0.12	-2.38	0.018	-0.50, -0.05
Constant	7.26	0.86	8.44	0.000	5.58, 8.95

Discussion

The relationship between metabolic and cardiovascular health has been well-studied in sedentary, urban, industrialized populations. Due to the role of adiposity in leptin levels, these levels are often found to be associated with BMI-associated cardiovascular complications. Thus, in individuals with a BMI greater than 30 kg/m^2, leptin levels are positively associated with myocardial infarction (Wallerstedt et al., 2004), hypertension (Wallerstedt et al., 2004; Sierra-Johnson et al., 2007), diabetes (Welsh et al., 2009), and coronary heart disease and stroke (Sierra-Johnson et al., 2007; Liu et al., 2010). The role of adiponectin in cardiovascular health seems to be a delicate balance. Along with leptin, adiponectin levels are associated with BMI-related cardiovascular disease, though the association is reversed (Hung et al., 2008). Low adiponectin levels are associated with hypertension, atherosclerosis, and ischemic heart disease (Mallamaci et al., 2002; Kumada et al., 2003; Meier & Gressner, 2004).

The results of this study support the associations found between these metabolic hormones and overall cardiovascular health, suggesting that there is a crucial relationship between metabolism and heart health that is inherent to human physiology, regardless of ecology, physical activity, or lifestyle. However, these results demonstrate the need for further research into the role of leptin and adiponectin in the development of cardiovascular *disease* itself, particularly in more variable contexts. CAC and epicardial fat are known to be very reliable predictors of cardiovascular disease. The current state of the literature suggests that CAC is positively associated with leptin levels (Reilly et al., 2004) and negatively associated with adiponectin levels (Maahs et al., 2005). Similarly, high amounts of epicardial fat are associated with high leptin and low adiponectin levels

(D'Marco et al., 2020). Yet neither metabolic hormone was associated with the presence or the extent of CAC or epicardial fat among the Tsimane and the Mosetan. These results demonstrate clear differences in two populations and begs the question about whether the mechanisms involved in the development of disease may not be directly related to these metabolic hormones, but rather indirectly.

Because these hormones are directly related to energy metabolism, it is more likely that their association with cardiovascular health is related to variation in energy expenditure. The trends observed in heavily industrialized societies may not adequately represent the complexity of metabolism. Studies in the United States show a defined relationship between body composition, cardiovascular disease, and metabolic hormones (Hou & Luo, 2011; Meier & Gressner, 2004). This is likely due to a wide range of factors including sedentary lifestyles, highly processed/high fat diets, and minimal disease/pathogen exposure. These confounding factors may be masking the extent of variation in metabolic physiology. It is possible that body "fat" may not be so simply associated with metabolic or cardiovascular health. Rather, total daily energy expenditure (regardless of body composition) and possibly immune regulation may be the keys in mediating cardiovascular complications. First and foremost, leptin and adiponectin are indicators of energy availability. The relationship between body fat and energy availability is not as simplistic as the current models suggest and thus begs further exploration.

A few foundational studies conducted among the Ache Amerindians of Eastern Paraguay demonstrated that ecological conditions and activity levels can significantly impact the strong relationship between body fat percentage and leptin levels that are

observed in American populations. These studies suggested that the relationship between body fat and leptin levels observed in American populations are not quite so simple because the American populations still had higher levels of leptin, even when controlled for body fat (Bribiescas, 2001, 2005; Bribiescas & Hickey, 2006). In fact, American long-distance runners with very low body fat percentages still had higher levels of leptin than did Ache individuals with higher body fat percentages (Bribiescas & Hickey, 2006). These studies clearly demonstrate that the current understanding of the relationship between body fat and metabolic hormones is potentially misunderstood.

The studies among the Ache were fairly limited by small sample sizes (highest n=33). This was likely due to the overall population size and geographical dispersion seen among the Ache. The present study is by far the largest study (n=1670) to explore the expression of metabolic hormones in a relatively smaller population with considerably different ecology, subsistence, and overall lifestyle. This study highlights the importance of exploring human physiology outside of the typical research contexts. Smaller, but highly variable populations can provide key insights into the extent of human variation and often draw into question the underlying assumptions about what is considered "normal" human physiology. Both leptin and adiponectin have been virtually unstudied in smaller, subsistence-based populations. Thus, the research in the Tsimane and the Mosetan substantially adds to the understanding of cardio and metabolic health variation. In order to address the global "obesity" and cardiovascular health crisis, research must understand the complete extent of human cardiovascular and metabolic variation. This demands that research must be conducted outside of the typical contexts where lifestyle, culture, and ecology may be masking physiological variation.

Conclusion

Cardiovascular disease contributes to 32% of all deaths worldwide. However, understanding the complexities involved in the development of CVD has been unfortunately limited to large, sedentary, industrialized populations. However, research is beginning to demonstrate that human physiology is far more variable than what is seen in these contexts. Therefore, this study sought to explore how the metabolic hormones (leptin and adiponectin) are associated with cardiovascular disease risk in the Tsimane forager-horticulturalists of lowland Bolivia. The results of this study suggest that while these hormones certainly play a crucial role in cardiovascular health, the relationship seen in industrialized societies with the presence of disease itself may not be as concrete. Future studies should seek to explore and disentangle the relationship between energy metabolism and cardiovascular health by exploring these relationships outside of the typical contexts.

CHAPTER 4

Interest in studying human physiological variation from a global perspective has increased over the last few decades. Studies have demonstrated that differences in ecology, population history, culture, discrimination, subsistence strategies, pathogen and parasitic loads can influence variation in human health and physiology (Ellison, 2017; Gurven & Lieberman, 2020; Konner & Worthman, 1980). Even still, most studies that attempt to employ a global perspective still remain within sedentary, urban, industrialized populations. Therefore, many anthropologists have taken on the task of working with smaller populations that are distant from markets, who generally differ from the lifestyle of the industrialized world (e.g., Gurven et al., 2017; Hill & Hurtado, 1995; Marlowe, 2010; McDade et al., 2007). However, this task comes with its own unique challenges.

Non-local researchers working in collaboration with populations living in market-distant areas can often mean the researchers will be navigating difficult terrain, language barriers, ethical considerations, and low access to quality medical equipment and supplies. This presents challenges to biospecimen collection and transport, obtaining proper consent, and minimizing potential negative impacts on the communities (Boerma et al., 2001). Research studies that explore these methodological and ethical complications could identify practical solutions for anthropologists. One such solution for the methodological issues surrounding sample collection and transport in market-distant populations has been the increasing use of dried blood spot (DBS) sampling for biomarker analysis (Mcdade et al., 2007; McDade, Aronoff, et al., 2021; McDade, Miller, et al., 2021). DBS sampling has many advantages when compared to venous blood sampling. The collection process is far less invasive to the individual participant,

involving only a pin prick of the finger. Following a venous blood draw, specimens must be centrifuged and refrigerated or frozen, all of which require electricity and specialized equipment which makes it difficult to conduct such studies outside of laboratory settings. These equipment and electricity requirements are not only a burden to conducting medical care in market-distant settings, but also means that most bio-behavioral research is conducted in urban dwelling populations, creating barriers to understanding human diversity. The transport and preparation procedure of the DBS cards involve far less expensive and cumbersome equipment, and they do not require the level of ambient temperature control that venous blood requires for storage and transport (Boerma et al., 2001).

The use of DBS sampling dates back to the 1960's when Dr. Roebert Guthrie used heel-prick blood spot samples to detect phenylketonuria in infants (Guthrie & Susi, 1963). Since then, DBS sampling has proven to be a useful tool in the nationwide screening of newborns for a number of treatable metabolic disorders (Mei et al., 2001). However, only recently has the implementation of DBS sampling been seen in population-based research (Boerma et al., 2001; Mcdade et al., 2007; McDade, Aronoff, et al., 2021; McDade, Miller, et al., 2021; Worthman & Stallings, 1994).

One benefit to DBS sampling is that it achieves the same sample quality as other more invasive methods. DBS collection papers are made from high-purity cotton linters. They are certified by the Clinical and Laboratory Standards Institute and undergo independent quality control by the Center for Disease Control & Prevention (CDC) (Mcdade et al., 2007; Mei et al., 2001). The DBS sampling method achieves the same level of precision and replicability as the standard methods of collecting blood (e.g.,

vacuum tubes & capillary pipettes) (Mei et al., 2001). The filter paper stabilizes most biomarkers in DBS, but the rate of sample degradation will vary case by case. For example, antibodies against the Epstein-Barr virus are stable in dried blood for at least 8 weeks in room temperature (McDade et al., 2000). However, the concentration of C-reactive protein only stable for more than 2 weeks at room temperature (TMcDade et al., 2004). The stability of most biomarkers in DBS provides flexibility for sample collection in field settings. However, it is advisable to refrigerate or freeze samples as promptly as possible after drying to minimize any chance of degradation. For long term storage, samples should be packed with desiccant and frozen in laboratory-grade freezers. While the filter paper does seem to offer a degree of protection against sample degradation for up to 6 freeze-thaw cycles, unnecessary freeze-thaw cycles should be avoided (Brindle et al., 2010; Mcdade et al., 2007).

Unlike standard blood sampling methods, the shipping requirements for DBS samples are relatively minimal, as they are not typically considered a biohazard. Most samples, unless they are known to contain an infectious or etiologic agent, are considered diagnostic specimens and should be labeled that way for shipment. The filter papers stored in sealable plastic bags with desiccant can be sealed in a secondary container and shipped domestically without any special permitting or packaging. For international shipments, the CDC will issue importation permits. However, these permits may only be required under certain circumstances. Shipping and importation guidelines are updated and available through the CDC website (http://www.cdc.gov/od/ohs/biosfty/biosfty.htm) (Mcdade et al., 2007).

The assay protocols for DBS samples are comparable to plasma or serum samples, with a few notable exceptions. Because the sample has been dried on filter paper, the samples first must be turned into a solution. A standardized hole punch is used to punch out discs of the DBS sample. These discs are then placed in an elution buffer overnight. During this process, the DBS is reconstituted into hemolyzed liquid whole blood. This can then be used in place of plasma or serum across multiple types of assay protocols.

Given the practicality of their use, it is no surprise that researchers working with market-distant populations have begun to use DBS sampling. However, transport conditions from the these areas to urbanized areas has presented its own unique challenge. The DB cards are transported in liquid nitrogen (LN2) dewars and due to the occasional rough conditions of road or water travel, the cards are sometimes at risk of direct exposed to the liquid nitrogen itself. While cards are protected in multiple layers of plastic bag and have desiccant, there is always a contamination risk during travel. This is a methodological challenge that is unique to anthropologists and other researchers working in market-distant areas and no study to date has explored whether or not this contamination could potentially alter the integrity of the sample.

The purpose of this study is to explore whether or not the exposure to liquid nitrogen could lead to degradation of the samples or the cross-contamination of multiple samples. We achieved this goal by conducting three experiments: 1) testing whether direct exposure to liquid nitrogen degrades samples 2) testing whether indirect sample contact while submersed in liquid nitrogen contaminates the samples and 3) testing whether direct sample contact while submersed in liquid nitrogen contaminates the samples.

Methods

DBS Sample Collection & Assay Protocol

The procedure for collecting DBS samples is relatively simple. A participant's finger is cleaned with alcohol and then pricked with a sterile and disposable lancet. This device is designed to deliver a uniform puncture that stimulates blood flow with minimal injury to the participant. The first drop of blood is to be wiped away with sterile gauze while the subsequent drops are applied to the filter paper. Once the samples have dried, they can be stacked and stored with desiccant in sealable plastic bags or containers (Mcdade et al., 2007). DBS samples were analyzed to determine C-reactive protein (CRP) concentration at the Arizona State University (ASU) Evolutionary Medicine and Biodemography laboratory via enzyme immunoassay (ELISA), using a protocol validated against the University of Washington lab responsible for NHANES data analyses (Kaplan et al., 2017). The samples used for this study were the standards (S1-S8) created for the CRP assay, in addition to 4 existing laboratory samples (Samples A-D), see Table 15.

Experiment 1: Sample Exposure to LN2

The first experiment sought to test whether direct exposure to liquid nitrogen could alter or degrade a sample. The DBS samples used were CRP assay standards (S2-S8, ranging from 0.316 ng/mL of CRP in S1 up to 20.2 ng/mL in S8) and 4 laboratory samples (A-D). Each were individually and directly exposed to LN2 for 4 minutes, or not exposed to any LN2 treatment, see Photograph 1. The standards and samples were then placed in elution buffer overnight and underwent CRP in-house assay protocol for development. These specimens were then compared to DBS cards collected at the same time that had not been exposed to LN2.

Table 15

Sample Characteristics

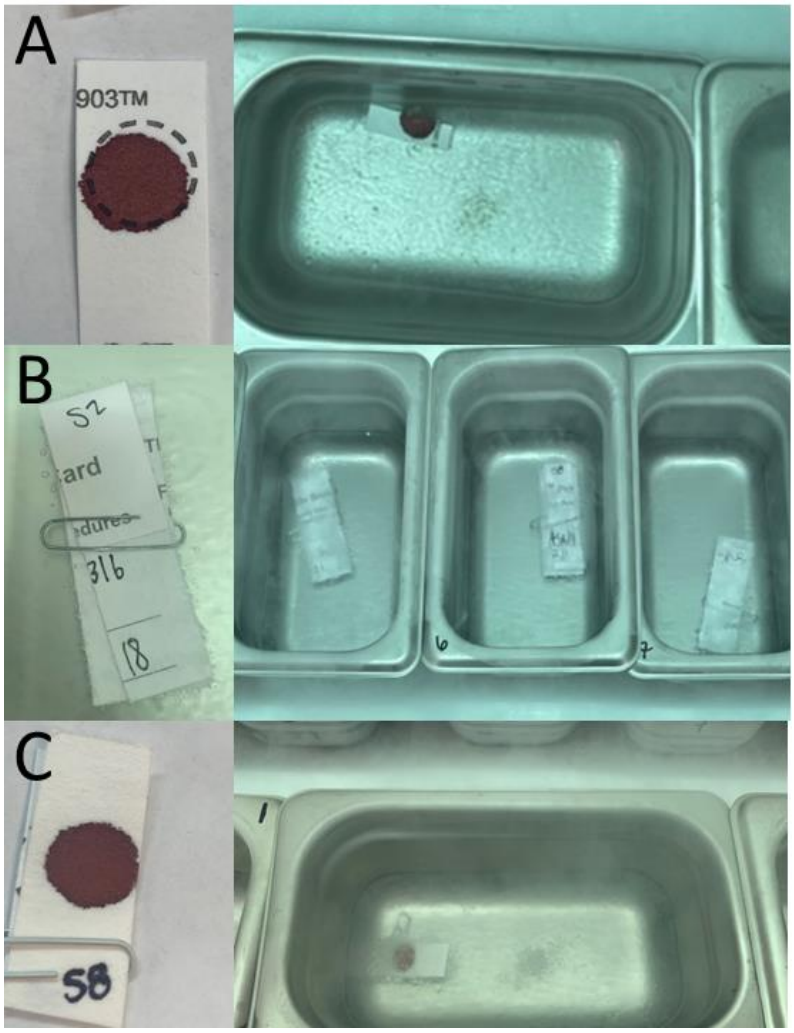
Standards	Estimated Concentration (CRP ng/mL)
Standard 1	0
Standard 2	0.316
Standard 3	0.631
Standard 4	1.262
Standard 5	2.524
Standard 6	5.048
Standard 7	10.1
Standard 8	20.2

Experiment 2: Indirect Sample Contact & Contamination

The second experiment sought to explore whether indirect sample contact during exposure could lead to cross-contamination of samples. The DBS samples used were the CRP assay standards (S2, 0.316 ng/mL and S8 20.2ng/mL) As the lowest standard point and the highest standard point, if there was to be any cross-contamination, it would be evident with this paradigm. Three laboratory samples (B-D) were also given indirect exposure to S8, again to create maximum possible cross-contamination. Sample A was left out due to insufficient volume. DBS cards were paper clipped together with no direct contact between the spots, see Photograph 1. They were then submerged in LN2 for 4 minutes. Standard 2 and Standard 8 were paper clipped together and each laboratory sample (B-D) was paper clipped to Standard 8. The standards and samples were then placed in elution buffer overnight and underwent CRP in-house assay protocol for development.

Experiment 3: Direct Sample Contact & Contamination

The third experiment sought to explore whether direct sample contact during exposure could lead to cross-contamination of samples. The DBS samples used were the CRP assay standards (S2 0.316 ng/mL and S8 20.2 ng/mL) and the 4 laboratory samples (A-D). Once again the lowest standard point and the highest standard point used to maximize any cross-contamination, and all lab samples were exposed to S8 to ensure maximum potential cross contamination. DBS cards were paper clipped together so that the blood spots were directly touching and then were submerged in LN2 for 4 minutes, see Photograph 1. Standard 2 and Standard 8 were paper clipped together and each laboratory sample (A-D) was paper clipped to Standard 8. The standards and samples were then placed in elution buffer overnight and underwent CRP in-house assay protocol for development.



Photograph 1. ***Layouts of Experiments 1-3. Experiment 1 (Image A): DBS cards were placed in LN2 for 4 minutes and then were compared to unexposed DBS cards to determine whether submersion in LN2 can influence biomarker values. Experiment 2 (Image B): DBS cards were paper clipped together, submerged in LN2 for 4 minutes, and then were compared to unexposed DBS cards to determine whether cross-contamination could occur during LN2 submersion. Experiment 3 (Image C): DBS cards were paper clipped together with the blood spots making direct contact. They were submerged in LN2 for 4 minutes and were then compared to unexposed DBS cards to determine whether cross-contamination could occur during LN2 submersion.***

Results

Experiment 1: Sample Exposure to LN2

The results suggest that submersion in liquid nitrogen had no significant impact on DBS values, see Table 16. This was evaluated two different ways; first by examining all standard curve points together in a regression model compared to the standard curve points that were not exposed, and secondly by individually comparing T-Tests for each individual standard point in LN2 versus no treatment separately. Regression results showed no impact of LN2 treatment on the standard curve (b=-.255, p=0.280, 95% CI = -0.733, 0.221). Secondly, there were no significant differences between any standard curve points, or any sample values, see Table 16.

Table 16

Two-sample t test for experiment 1

Sample	Value LN2 (95% CI)	Value No Tx (95% CI)	p value
STD 2	0.251 (-0.14, 0.64)	0.441 (-1.92, -2.80)	0.420
STD 3	1.176 (-2.06, 4.41)	1.235 (1.19, 1.28)	0.838
STD 4	1.195 (-2.81, 6.72)	2.125 (-5.39, 9.64)	0.828
STD 5	3.039 (-1.74, 7.82)	2.527 (-2.66, 7.72)	0.453
STD 6	3.381 (2.64, 4.12)	3.063 (2.00, 4.13)	0.090
STD 7	2.615 (1.95, 3.28)	4.208 (-3.94, 12.35)	0.132
STD 8	2.980 (1.26, 4.70)	3.586 (2.28, 4.89)	0.070
Sample A			
Sample B			
Sample C	0.558 (-1.67, 2.79)	0.503 (0.37, 0.64)	0.784
Sample D	2.468 (-10.87, 15.80)	2.444 (-10.39, 15.28)	0.989

Experiment 2: Indirect Sample Contact & Contamination

Overall, the results suggest that there is no likely contamination of the two DBS cards together in the same liquid nitrogen. In each of these cases, as lab sample was exposed to the highest standard curve point (beyond normal human levels of CRP), and there was little evidence of any cross contamination. This was evaluated by individually comparing T-Tests for each individual standard and sample. There was no significant impact of indirect contact and submersion in LN2 for either of the standards, or for samples C & D, see Table 17. There was a slight but significant impact observed for sample B (p=0.011). However, it is probable that an error occurred during preparation of the assay or that an error occurred during the collection of the sample.

Table 17

Two-sample t tests for experiment 2

Sample	Value LN2 (95% CI)	Value No Tx (95% CI)	p value
STD 2	0.929 (0.81, 1.05)	0.877 (0.81, 0.95)	0.385
STD 8	1.242 (1.15, 1.33)	1.227 (1.19, 1.26)	0.316
Sample B	1.063 (0.96, 1.16)	0.985 (0.95, 1.01)	0.011
Sample C	0.851 (-0.06, 1.77)	0.936 (0.93, 0.94)	0.361
Sample D	1.097 (-0.84, 3.03)	1.061 (-0.78, 2.90)	0.882

Experiment 3: Direct Sample Contact & Contamination

The impact of direct sample contact on contamination for experiment 3 was evaluated by comparing T-Tests for each individual standard and sample. There was a slight increase in concentration for the Standard 2 blood spot when it was directly

touching the Standard 8 blood spot (~13% higher, p=0.286). There was no effect of direct contact for Standard point 8 when touching Standard 2 (p=0.198). There were no significant differences for any of the participant blood spot cards when next to Standard 8, though Sample A closely approached significance (p=0.051), see Table 18. Note that in table 4 there is no uniform directionality of specimens based on treatment; we would expect that in contamination was occurring that all S2 points would be higher when exposed to S8, and similarly all S8 points to be lower when exposed to S2- this was not the case.

Table 18

Two-sample t tests for experiment 3

Sample	**Value LN2 (95% CI)**	**Value No Tx (95% CI)**	**p value**
STD 2	0.868 (0.83, 0.90)	0.760 (0.67, 0.85)	0.0286
STD 8	1.172 (1.12, 1.22)	1.132 (1.08, 1.18)	0.198
Sample A	0.710 (0.27, 1.15)	0.473 (-0.31, 1.26)	0.051
Sample B	0.968 (0.75, 1.18)	0.924 (0.85, 0.99)	0.159
Sample C	0.736 (-0.87, 2.34)	0.863 (0.85, 0.88)	0.498
Sample D	1.025 (-0.90, 2.95)	0.981 (-1.09, 3.05)	0.854

Conclusion

The first experiment suggests that liquid nitrogen exposure has no impact on sample integrity, indicating that even a relatively extended period of direct liquid nitrogen exposure (more than would be experienced during market-distant field work) is unlikely to contaminate or impact DBS values. The second experiment suggests that even when two cards are both submerged together into liquid nitrogen, it is unlikely that cross-

contamination would occur. As this is how must specimens are stored, this suggests that there is little danger that liquid nitrogen could re-constitute or cross-contaminate samples. From a physics standpoint, liquid nitrogen is -195.8 C, and thus there is little movement even at the molecular level. Lastly, the third experiment suggests that it is possible that if two blood spots are directly touching, then sample contamination may occur. That said, not only would these conditions never exist during any sort of field specimen collection, but even then, contamination was mild and inconsistent.

Overall, the results of this study suggest that despite the challenges of sample collection and shipment from market-distant areas, DBS sampling is a flexible and stable alternative to standard blood sampling techniques. The three scenarios presented in this study represent the most extreme examples of liquid nitrogen exposure. It is unlikely that a DBS card would ever become completely submerged in liquid nitrogen, unless a tank was somehow poured out or put upside down. Even still, complete submersion in liquid nitrogen, whether the individual sample alone or two samples together, does not appear to impact sample integrity. Only the most extreme example, where two blood spots were in direct contact with one another, showed any changes to sample values, and even then, the modifications were minor and inconsistent.

Therefore, researchers who work with market-distant populations should consider using DBS sampling as an alternative to standard techniques because it is A) less invasive to research participants B) more practical to store and transport & C) achieves the same sample quality as standard techniques. Despite the stability of DBS samples in liquid nitrogen, proper precautions to avoid contamination in the field should still be taken. The results of this study offer support to the growing trend in population-based of using DBS

sampling. DBS sampling is a practical, easier solution to standard techniques of blood sampling because of the simplicity in the collection and storage of specimens, making it an ideal method for researchers.

This manuscript fills an important void in the literature - while there are hundreds of scientific papers written by researchers that collect specimens on dried blood spot cards across disciplines from biomedicine, biology, anthropology, and demography, this is the first manuscript to assess the potential impacts of liquid nitrogen exposure on specimen stability or cross-contamination. These results suggest that DBS samples are robust to several different types of liquid nitrogen contamination, further re-enforcing the value of DBS specimen collection. Having DBS in the research toolkit can help democratize science, by increasing not only access to medical diagnostics in market-distant settings without access to centrifuges and a cold chain, but also by expanding our knowledge of global studies of health, which in the past have been largely focused on industrial urban populations.

CHAPTER 5

The very broad goals of this dissertation were to: 1) expand on the knowledge of human metabolic variation 2) to demonstrate the necessity for research outside of the typical sedentary urban research contexts and 3) to highlight key areas for future research. Each and every chapter focused on some or all of these goals.

Summary of Chapter 1

The first chapter explored the relationship between metabolic hormones, food insecurity, and depression. One evolutionary hypothesis argues that depression is a strategy that serves to regulate energy allocation, especially when faced with high levels of parasites and pathogens in food-limited environments (Nesse, 2000). Because the hormones leptin and adiponectin play a crucial role in metabolic function and energy availability (Meier & Gressner, 2004), they therefore may be important physiological mediators of the effect of nutritional status on depression. Both hormones originate in adipose tissue; leptin levels generally increase with adiposity, while adiponectin decreases with adiposity (Flier, 1998; Maeda et al., 1996). However, these hormones have not been well-studied in contexts of food insecurity, high levels of physical activity, high parasite and pathogen loads, and low body fat.

In this first chapter, I explored the relationships between leptin and adiponectin, self-reported food insecurity, and depression among Tsimane forager horticulturalists of lowland Bolivia. Leptin and adiponectin levels were quantified among 148 Tsimane adults (mean age= 58, range= 36-92, 49% female) using enzyme immunoassays. 34% of respondents surveyed reported recent concerns about food insecurity. Log leptin levels were negatively associated with reported food insecurity (β= -0.372, P= 0.02) when

adjusting for age, body fat, BMI status, and sex. Log adiponectin levels were negatively associated with depression scores (β= -0.302, p=0.004). However, there were no associations between adiponectin and food insecurity, nor leptin and depression scores. These results suggest that leptin may be a good proxy of short-term food insecurity, even in a low body fat population.

Summary of Chapter 2

Cardiovascular diseases (CVDs) contribute to 32% of all deaths worldwide (WHO, 2017). Both leptin and adiponectin are associated with various indicators of CVDs. However, understanding the complexities involved in the development of CVD has been unfortunately limited to white, WEIRD populations. Foundational research has demonstrated that human physiology is far more variable than what is seen in those contexts (see Ellison, 2017; Gurven & Lieberman, 2020; Konner & Worthman, 1980; Kaplan et al., 2017; Trumble & Finch, 2019).

In this second chapter, I explored how the metabolic hormones are associated with CVD risk in the Tsimane. Leptin and adiponectin levels were quantified among 1,607 Tsimane adults (mean age= 60, range= 32-94, 51% female) using enzyme immunoassays. Coronary Artery Calcium, Epicardial fat, and liver density, and aortic valve and thoracic aortic calcium were measured with an electrocardiogram (ECG)-gated CT imaging using a 16-detector row scanner. A licensed radiological technician acquired a single, ECG-gated scan under the supervision of a team cardiologists. A core laboratory performed calcium scoring of the coronaries using semi-automatic software.

Leptin was found to be positively associated with systolic blood pressure (b=2.39, p=0.000, 95% CI= 1.31, 3.48) and hepatic steatosis (b=1.04, p=0.036, 95% CI= 0.07,

2.00). Leptin was not associated with the odds of having any thoracic calcium but was positively associated with the extent of any thoracic calcium (p=0.001, 95% CI= 1.08, 1.32). Leptin was found to be negatively associated with liver density (b= -2.1, p=0.000, 95% CI= -2.71, -1.55). Additionally, low leptin levels were associated with the odds of having no aortic valve calcium (p=0.018, 95% CI= -0.50, -0.05). Adiponectin closely approached positive significance with diastolic blood pressure (b=0.65, p=0.060, 95% CI= -0.03, 1.33). Adiponectin was not associated with the odds of having aortic valve calcium but was positively associated with the extent of any aortic valve calcium (p<0.001, 95% CI= 1.36, 2.32).

The results of this study support the associations seen between these metabolic hormones and overall cardiovascular health in typical research contexts. This suggests that there is a crucial relationship between metabolism and heart health that is inherent to human physiology, regardless of ecology, physical activity, or lifestyle. However, these results demonstrate the need for further research into the role of leptin and adiponectin in the development of cardiovascular disease itself, particularly in more variable contexts.

Summary of Chapter 4

Conducting research in collaboration with populations living in areas that are distant from markets can mean researchers often find themselves navigating unfamiliar and difficult terrain, language barriers, ethical considerations, and limited access to quality medical equipment and supplies. This presents challenges to biospecimen collection and transport, obtaining proper consent, and minimizing potential negative impacts on the communities (Boerma et al., 2001). A solution to the methodological issues surrounding sample collection and transport in these populations has been the

increasing use of dried blood spot (DBS) sampling for biomarker analysis (Mcdade et al., 2007; McDade, Aronoff, et al., 2021; McDade, Miller, et al., 2021). DBS sampling requires less equipment, less money, and is less invasive. However, transport conditions from these market-distant areas to urbanized centers has presented its own unique challenge. The DBS cards are transported in liquid nitrogen (LN) dewars and due to the occasional rough conditions of road or water travel, the cards are sometimes at risk of direct exposed to the liquid nitrogen itself.

This third chapter explored whether or not the exposure to LN could lead to degradation of the samples or the cross-contamination of multiple samples. We achieved this goal by conducting three experiments: 1) testing whether direct exposure to liquid nitrogen degrades samples 2) testing whether indirect sample contact while submersed in liquid nitrogen contaminates the samples and 3) testing whether direct sample contact while submersed in liquid nitrogen contaminates the samples.

The results of the first experiment suggested that LN exposure has no impact on sample integrity, which indicated that even a relatively extended period of direct LN exposure is unlikely to contaminate or impact DBS values. The second experiment suggested that even when two cards are both submerged together into LN, it is unlikely that cross-contamination would occur. As this is how must specimens are stored, this suggests that there is little danger that liquid nitrogen could re-constitute or cross-contaminate samples. Lastly, the third experiment suggested that it is possible that if two blood spots are directly touching, then sample contamination may occur. However, even then, contamination was mild and inconsistent.

This last chapter demonstrated that having DBS in the research toolkit can help democratize science, by increasing not only access to medical diagnostics in market-distant settings, but also by expanding our knowledge of global studies of health, which in the past have been largely focused on industrial urban populations.

Implications

The work presented in this dissertation has expanded the knowledge about the extent of human metabolic variation. While much of the data regarding leptin and adiponectin did fall in line with the current state of the literature, some of the results also indicate that certain trends are not observed in this particular population who do not fit within the white, WEIRD paradigm. This demonstrates the need to evaluate human variation and health under in multiple ecologies, not just urban research settings. The Tsimane are one population of many whose lifestyle, culture, ecology, and history differ substantially from typical research contexts. Future research should explore these metabolic hormones in a wide variety of contexts. Given how critical metabolism for human survival, it is shocking that these key metabolic hormones have not been better studied in multiple geographic contexts. This dissertation is a first step towards a better understanding of the global variation in human biology.

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APPENDIX A

IRB DEFERRAL

DEFERRAL

Benjamin Trumble
CLAS-SS: Human Evolution and Social Change, School of (SHESC) 480/965-1394
trumble@asu.edu

Dear Benjamin Trumble:

On 2/17/2022 the ASU IRB reviewed the following protocol:

Type of Review:	IRB Site
Title:	Testing Hypothesized Pathways Linking Infection, Physical Activity, Apoe Genotype, And Biological Sex To Low Dementia Prevalence And Reduced Brain Atrophy In Two Native American Populations
Investigator:	Benjamin Trumble
IRB ID:	STUDY00015596
Funding:	Name: HHS-NIH: National Institute on Aging (NIA), Grant Office ID: AWD00032641, Funding Source ID: 2 R01 AG054442-03
	AWD00032641;
	AWD00032641;
Documents Reviewed:	• CITI Training, Category: IRB Protocol; • Grant, Category: Sponsor Attachment; • Jaclyn Thomas CITI, Category: IRB Protocol; • Local Context, Category: IRB Protocol; • UCSB approval letter, Category: IRB Protocol; • UCSB approved protocols, Category: IRB Protocol;

The ASU IRB deferred review and oversight of this project to UCSB IRB and the associated IRB protocol number is 28-21-0788.

REMINDER – Effective January 12th 2022, in-person interactions with human subjects require adherence to all current policies for ASU faculty, staff, students and visitors. Up to-date information regarding ASU's COVID-19 Management Strategy can be found here. IRB approval is related to the research activity involving human subjects, all other protocols related to COVID-19 management including face coverings, health checks, facility access, etc. are governed by current ASU policy.

Sincerely,

IRB Administrator
cc:
Stephanie Koebele
Zane Jacobs
Jaclyn Thomas
Melissa Wilson
Julie Lawrence
Caden Elizalde
India Schneider-Crease
Kenneth Buetow
Angela Garcia
Noah Snyder-Mackler

APPENDIX B

DEPRESSION SURVEY

¿Me` adac jäm`dye`ya` cuti yoqui mi? ¿Anic där tari jäm`dye`ya` mi?	¿Me` adac jam jun` anicjeyacsi yoctyi` mi? ¿Me` buty ĉuti mi cavijcan in judyeya` cächä`chun in?
¿Me` adac mi jam jaijve tari?	¿Me` buty mo'ya yoc ĉoshdye mi? ¿Coshij buty mi uyaya are` muju`ĉha` jäm` jam jeñej räjcan? ¿Coshij buty mi camanacvajoij jam chu`chuij are` jam jäm` coshij mi?
¿Ñäñiti buty mi ĉui` me` juijya` jam jäm` ĉuti mi?	¿Aty jam säcsedye`dyeij mi? ¿Jam ma`je` säcsedye`dyeij mi, are` dai` ma`je` säcsi mi?
¿Me` buty mi ĉuti jam carij bä`yi? ¿Ma`je` buty mi säñi ĉuti? ¿Ma`je` ujati ĉuti mi? ¿Ma`je` jun`jiti ĉui` mi?	¿Me` adac mi aty jam anicjiti cui` are' jäqui`ves yocsi` dyijyedyem?
¿Mi ĉuti jam jun` tupuj me`je` carijtacdye` jam juijya` jen`sim, tse`sim`, viyas mi, jäyes mi, atas mi judyeya` tsedyes mi? ¿Mi ĉuti muju`cha` aty jam jäm` jam jeñej jiquej? ¿Mi ĉuti aty jam jedyedyety mi?	¿Me` buty mi ĉuti aty jam tupuj yutaqui peyacdye cui'si mi?
¿Me` adac mi ĉuti itsi` seyedye' mi jedye` ĉan ĉui` (yeja` dyijtucsi pen`dyem, majmadye` perota, se`vacdye` musica judyeya` carijtacdye`)? ¿Ĉuti mi aty jam yedye`dyeij mi judyeya` aty jam jun`bu`yi mi?	¿Me` buty ĉuti mi choco`tac? ¿Ĉuti buty mi jemoñe so'mac va`tacdye` mi are mo'ya ñibe`betacdye` mi jam tsan jemoñes mi?
¿Cuti no`bi`bij mi, cajotyiti mi judyeya` jam yedye`dyeij mi? ¿Mi aty cuti aty jam fer judyeya` dyä`cäij mi?	¿Me` adac jam jaijve faĉoij mi? ¿Me adac dam cavin faĉoij mi, jam dam` jäm`yi ĉuti mi?
¿Me` buty mi carij dyijyi mi judyeya` peye' dyijyedye` mi?	¿Ju`ñis ĉui` momo` dyijyedye` jo`no` ĉan tsoi` mi, anic pucmun dyijyim judyeya` jam ma`je` yaquinjoban mi?
¿Me` adac mi jäm`dye`ya` nocho`choij` mi? ¿Me` adac mi där yoquij judyeya` noi`yi mi? ¿Me` buty mi ĉuti jun` ra` ĉui` jiyi` mi?¿Mi ĉuti jam jun` jäm` judyeya` jam jun` tupuj chu`chuij mi?	¿Itsij säcsedye'?