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Deconstructing Normal: Leptin Variation Across and Within Population

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ABSTRACT

Deconstructing Normal: Leptin Variation Across and Within Population

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Since Franz Boas' work in the early 20th century understanding biological variation and plasticity has been one of the aims of Biological Anthropology research. This research has led to the recognition that deriving biological knowledge primarily from western industrialized samples provides a limited and potentially skewed view of human physiology. As such, this research focuses on leptin, a recently identified hormone that has largely been studied in western and industrialized samples. Leptin is a hormone like peptide secreted in proportion to adiposity and is involved in energy regulation. Specifically, this research examines cross-cultural variation in the hormone leptin. It examines and consolidates the available literature on leptin and draws on that literature to investigate the broad variation across a variety of populations. Then it takes an in-depth look at leptin within two populations, the Tsimane' and the Philippines. The Tsimane' are a population of hunter-gatherer horticulturalist in the early stages of market integration while the Philippines is a population that has been experiencing the nutrition transition.

The broad cross-cultural analysis shows a fair amount of variation in leptin levels across populations, only some of which is explained by variability in adiposity levels. Fat mss compared to percent body fat explains more of the variation in leptin levels; however the difference is quite small. Sexual dimorphism in leptin levels is consistently observed within and between populations, suggesting a species wide characteristic. Lifestyle patterns may have some influence on leptin levels, with rural and subsistence based populations tending to have lower values, but until more data is available it remains inconclusive.

The Tsimane' show very low leptin levels compared to other populations and show a high degree of continuity with adolescent Tsimane' samples previously analyzed. However, some of these results may be the due to the collection method used (bloodspot instead of venipuncture). The Filipino adults show moderate to high leptin levels compared to other populations and show a low degree of continuity with adolescent Filipino data (who have low leptin levels). In both population pregnant women show elevated levels of leptin, a pattern consistent with data from other studies.

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Chapter 1: Introduction

In this dissertation I present the results of research examining between- and within-group variation in the hormone leptin. Leptin is a hormone secreted in proportion to adipose tissue which plays an important role in energy regulation. This research provides data on leptin that challenges conceptions of human biology extrapolated primarily from limited population data (i.e. US and European) that purport to represent the full variability of the human species. Leptin's recent identification makes it an ideal candidate for a study of this kind, as research and discourse on its function and variability have only been undertaken in the last 20 years. This introduction will discuss the theoretical issues that drive this dissertation work and will provide an outline of this dissertation's content.

One of the hallmarks of anthropology has been its interest in variation. In early anthropology this started solely as an examination of the other, targeting cultures and societies that were markedly different from those of the researchers. Much of the research on biological characteristics at this time (late 19th/early 20th century) focused on the fixity of types and attempted to elucidate differences between (racial) types (Johnston and Little 2000). This typological thinking was challenged in the early 1900's, most notably by Boas' work on head shape in immigrants (Boas 1911). This recognition of plasticity increased anthropologists' interest in not just between-group biological variations but also within-group variation. In the 1960's the Human Adaptability Component of the International Biological Program greatly increased the amount of research focused on human biological variability (Little and Kennedy

2010; Schell 1995; Lasker 1969). Further, beyond simply documenting differences, post-1960's research has taken a greater interest in explaining why such differences exist and has tried to frame their role in a larger theoretical frameworks (e.g. evolutionary, ecological, political-economic). As biological anthropology has developed as a science and has become more theoretical and explanatory the tools in which it uses have also developed, becoming increasingly more sophisticated.

Since the 1980's there has been an increasing movement towards the use of hormones and other molecular biomarkers in studies of human variation (Campbell 1994). Such methods can be useful in detecting functionally important biological variability not reflected in broader, more apparent phenotypic expressions, such as subtle changes in fecundity (Ellison 1994), in elucidating biological pathways through which physiology interacts with social, cultural, ecological factors (Crimmins and Seeman 2001), and in providing objective health and physiological measures. Much of this interest has been aided by technological advancements, including increased affordability and the development of more field-friendly collection methods, including salivary (Ellison 1994), urinary (Lasley, Mobed, and Gold 1994), and bloodspot methods (McDade et al. 2007; Valeggia 2007; Worthman and Stallings 1994, 1997). Early work in reproductive ecology was one of the first examples of a field to benefit from these methods, when it was shown that progesterone levels are higher in westernized countries compared to populations in more subsistence-level contexts (Ellison 1994). Such differences are not immaterial, as relying on the high progesterone levels in westernized women as the basis for

hormonal contraceptives, potentially increasing side effects and decreasing use in populations with much lower levels (Vitzthum et al. 2004).

This work brought to the forefront the idea that the biology of humans in westernized countries may be a poor model for the biology of humans that existed for most of our species' history. Eaton and colleague's 1994 paper on women's reproductive cancers highlighted that these differences between groups, in this case differences in reproductive life histories, could have profound effects on health outcomes, with individuals living under industrialized conditions having increased disease expression. Similar ideas have been articulated in Margaret Lock's work on menopause in Japan, critiquing the generalizations made by the medical community based upon a very narrow population sampling, suggesting that western woman may in fact be anomalous in many of their characteristics (Lock and Kaufert 2001). Lock instead suggests the idea of *local biologies*, where biological variation is "a product of individual lived experiences in specific environmental, historical, and sociopolitical contexts" (Lock 2012: 25). Additional critiques of westernized-centric models have recently been expressed in Joseph Henrich and colleagues's paper on WEIRD (Western, Educated, Industrialized, Rich, and Democratic) populations, where he highlighted the uniqueness of westernized cultures and their limited nature as models of human behavior, a viewpoint that could just as easily be discussing their roles as models of human biology (Henrich et al. 2010). Similar critiques within anthropology have been raised concerning conclusions drawn from captive primate studies (Boesch 2010; Leavens et al. 2010) or from few primate populations during brief time periods (Strier 2009) and how limited such results may be when trying to understand primate species as a whole. Only through a

comprehensive study of a species across various ecological contexts can we truly start to make conclusions about that species.

Similar to the inappropriateness of the western world as a reference model for human biology, the construction of "normal" biology through the use of animal models (particularly rodents) may also create inappropriate models that are often used uncritically and may provide results that are unlikely to be replicated in human populations (Mestas and Hughes 2004). For instance, a recent examination of immunological publications shows an extreme bias towards animal models in a number of major journals, despite the fact that conclusions drawn from this research may not ultimately be applicable to human pathophysiology (Hayday and Peakman 2008). On a most basic level, animals models have been criticized and cautioned by the fact that in a number of important ways mice physiology may differ from that seen in humans (Sarikonda et al. 2009) and much of the research that is done can be characterized as being of poor quality (Bracken 2009, Pound et al. 2004). Mouse models in particular have been criticized for their use of isogenic populations that poorly represent the substantial variability seen in non-laboratory genetic backgrounds as well as the complex genetic (and environmental) interactions that result from this (Williams et al. 2004). The fact that mouse life history (short-lived) and ecological conditions (ground dwelling) that they are adapted is radically different than human's has also been a point of criticism (Davis 2008, Mestas and Hughes 2004). Indeed a recent article highlighted the lack of systematic studies evaluating the appropriateness of mouse models and how human genomic responses to acute inflammatory stresses are poorly reproduced in such models (Seok et al. 2013).

The recent identification of the hormone leptin and the surge of research that followed provide an excellent opportunity to explore some of the issues discussed above. The application of problematic models in the attempt to understand leptin and the resulting incorrect conclusions exposes the potential of the dangers of using such models. This started with early conclusions drawn from results seen in the *ob*/*ob* strain of mice, with the administering of leptin leading from an obese phenotype to a lean one (O'Rahilly 1998). This led people to believe the leptin might prove to be a "cure" for obesity (e.g. Byravan 1996). This idea was quickly proven not to be the case in humans or mice, showing that with increasing obesity leptin levels increase and that exogenous leptin administration were largely unsuccessful at promoting weight loss (Huckshorn et al. 2000, Pelleymounter et al. 1995). The reason for the original erroneous conclusion is multifaceted, stemming from theoretical issues and the use of animal models.

For the most part views and research on leptin have taken an obesity centric view of studying the hormone. The earliest views were focused primarily on its role as a therapeutic agent in curing obesity. When that goal failed to materialize much of the research was still centered on obesity and the idea of leptin resistance as causative agent in obesity. A more evolutionary and ecologically oriented view of leptin approached the hormone from a different perspective, as an anti-starvation hormone, operating for most of human history at relatively low levels and functioning to help activate the body's defenses and energy saving mechanisms in the face of decreased energy availability (Flier 1998, Ahima et al. 1996). Leptin's role has been hypothesized beyond simply preventing starvation to one that may play a key role in mediating

life history tradeoffs that occur under conditions of finite energy (Worthman 2003), including mediating energy needs for immune function (Carlton et al. 2012; Mcdade 2003) and reproduction (Loucks 2007).

Much of the research done on leptin has been done using animal models, primarily mice. These models have shown some similarities to leptin's action in humans, particularly the hyperphagia and lack of reproductive development (e.g. puberty) seen in both the *ob*/*ob* mouse phenotype and the leptin deficient human phenotype should be noted. However, we now know that a number of important differences exist between humans and mice in relation to leptin, even when keeping our comparisons to those with similar genetics deficiencies to the *ob*/*ob* mice (see Table 1.1 for comparisons). With leptin's role in anti-starvation this makes sense that there would be differences considering mice must eat ~50% of their total energy content per day, while humans eats about 2% of their total energy content per day (Himms-Hagen 1999). Thus, food scarcity requires much more action and can be much more devastating in the mouse. This is one of the reasons humans don't down regulate body temperature and enter a state of torpor in response to acute food scarcity. Leptin may play a key role in this in mice, where ob/ob mice down regulate body heat production and then up regulate it when given leptin (Pelleymounter et al. 1995). Problems then may arise when specific leptin related traits are presented as occurring in humans, but then the citations given are often only for rodent studies (e.g. Fietta 2005).

The problematic use of mice models in understanding leptin is exemplified by the contention that leptin acts to raise energy expenditure. A number of studies have reported that

methodological problems in studies of mice that incorrectly adjust for total body weight, rather than lean mass have been argued to created difference where none exist (Butler and Kozak 2010, Himms-Hagen 1997). Additionally when given pharmacological doses of leptin increase in energy expenditure is seen in *ob*/*ob* mice (normalized to levels resembling controls) but no such increase is seen in genetically normal control mice (Pelleymounter et al. 1995). However, in calorie restricted mice, leptin administration appears to prevent decreases in energy expenditure (Halaa et al. 1997). Therefore, the idea of "leptin raising energy expenditure" should only be applied to *ob*/*ob* mice or food-restricted mice, not humans or mice under normal conditions (Himms-Hagen 1999). Thus, not only are the conclusions problematic because of methodological problems, but the reliance on genetically unique models (*ob*/*ob* mice) lead to inaccurate conclusions about normal mouse physiology that has then been erroneously carried over to humans.

This is not to say that mouse models do not play an important role in understanding human physiology. Much research, particularly experimental studies, relies on the control and ability to manipulate variables that animal models offer. The critique here is that all too often such models are used uncritically. In a similar vein drawing conclusions solely from westernized populations has the potential to lead to inaccurate conclusions about other populations or the human species as a whole. Research in the US may be convenient and does not face many of the challenges anthropological work does, but the conclusions drawn should be viewed as representing a specific local biology, one created as a result of the cultural and ecological context in which it occurs. Work by Bribiescas (2005) was one of the first studies to highlight this,

showing that at equal levels of body fat Ache woman have much lower leptin levels than women in the US and in fact Ache levels more closely resemble US women diagnosed with Anorexia nervosa (and very low body fats). Given how important energy regulation is for life history and leptin's potential role as a mediator, exploring leptin in contexts with different ecological/cultural context that impact energy availability as well as life histories patterns becomes even more essential.

This dissertation then is an attempt to move past westernized and animal models of leptin function and add to the small amount of literature available on leptin in non-westernized context. The two primary objectives of this study are to examine the cross-population variation in leptin and to add to the current available literature and understanding of leptin in two non-western populations. The Tsimane not only experience energetic stressors that may be closer to those met under conditions of the evolution of leptin's function in the human lineage, but also because they provide a good contrast to the conditions seen in WERID populations, with their own local biologies created under unique environmental, historical, and sociopolitical contexts. The data from the Cebu Longitudinal Health and Nutrition Survey (CLHNS) provides another contrast, as individuals from this population exist in a range of socio-ecological and economic conditions, circumscribed within the context of the development of a large metropolitan area and its immediate periphery that is currently undergoing the nutrition transition. Past research has shown adolescents from Cebu City to be lean while their adult mothers have increasing rates of overweight and obesity (Kuzawa et al. 2007, Adair 2004). Another benefit of the new leptin data from populations investigated within this dissertation is that earlier data has been previously presented from children and/or adolescents from these populations (Kuzawa et al. 2007,

Sharrock et al. 2008). Data from these two populations (Tsimane' and Filipino) allows not only cross-cultural comparisons, but also allows comparisons to be made to younger age groups within those specific populations.

Outline of the dissertation

This last section provides a brief overview of the dissertation, which is composed of seven chapters. This chapter has discussed some of the theoretical issues driving this dissertation and provides a structural overview of the dissertation.

Chapter 2 provides a review of leptin literature and analyzes current data on leptin cross-population variability. The first objective of this chapter is to provide a thorough examination and synthesis of the available literature on leptin. This review provides a discussion of leptin's identification and recent history, leptin physiology, and a discussion of leptin's function. Then the review focuses on leptin variability and causes of that variability.

Chapter 3 examines the available population data on leptin levels. First methodological issues with measuring leptin and comparisons between populations are discussed. Then this chapter takes a cross-cultural look at the variability in leptin levels from a variety of studies taken from the literature where leptin levels were reported as well as body fat percentage and fat mass (reported or calculated). Finally an analysis based upon a broad lifestyle factor is conducted as a way to explain the observed variability.

Chapter 4 provides relevant background information on the Tsimane', with whom over a year of field research was conducted. I start this chapter by providing a historical overview to better contextualize modern Tsimane'. I then discuss Tsimane' political involvement, village

life, and describe basic ecological conditions. Finally I go into more detail on means of subsistence, demographics, and market involvement.

Chapter 5 is an examination of anthropometric and leptin data in Tsimane' adults. The leptin data in this chapter was collected and analyzed using a bloodspot method adapted by myself and colleagues at Northwestern (Miller et al. 2006), utilizing the sophisticated, field friendly techniques developed within anthropology. After providing background on Tsimane' anthropometrics from children and adults and reviewing the research on leptin conducted previously in Tsimane' children and adolescents, I present methods for fieldwork conducted among the Tsimane' from December 2004 to March 2006. I present general descriptive and anthropometric data, test for sex differences, put my results in a comparative framework, and test for predictors of leptin that operate independent of adiposity. The discussion in this chapter examines the results in comparisons to those seen in Tsimane' children and adolescents and discusses their larger significance.

Chapter 6 is an examination of adult anthropometric and leptin data in Filipino adults. This chapter begins with a discussion of the research on leptin and anthropometrics previously conducted in Filipino adolescents as part of CLNHS. The analysis presented here examines leptin and its relationship to various adiposity measures, sex differences, and differences between pregnant and non-pregnant woman. I then discuss this chapter's findings in comparison to those found in adolescents.

Chapter 7 is the conclusion, providing a general discussion of the results of the research presented in this dissertation. Specifically, I discuss how the Tsimane' and Filipino data

compare to each other and to the world wide data presented in chapter 3. Finally, I discuss the theoretical and methodological implications of the results presented in this dissertation.

Chapter 2: Background on Leptin

Since leptin's initial discovery in 1994 there has been a tremendous growth of scientific literature concerning the hormone. Leptin has been identified as a product of the *ob gene* and secreted primarily by white adipose tissue. It has played a pivotal role in changing our understanding of adipose tissue, which has traditionally been viewed largely as a passive storage tissue, whereas now it is seen as a very active endocrine organ. A wide range of physiologic functions have been proposed for leptin in humans and generally these roles can be grouped under two headings: 1) regulating energetic status and 2) acting as an energetic indicator to various physiological processes, such as reproduction (Himms-Hagen 1999).

The focus of this chapter is to provide background information and a review of the pertinent literature relating to the hormone leptin, its function, and its variation in adult humans. Where considered relevant, animal models will also be discussed, with an understanding of the problems of such models previously outlined in the introduction.

Early History:

Research on the effects of leptin started long before the specific molecule was isolated and described. In the 1950s, researchers began studying what later became known as the *ob*/*ob* (obese) mouse (Ingalls et al. 1950) and the *db*/*db* (diabetes) mouse (Hummel et al. 1966), both of which were of interest due to their morbidly obese nature. It was later hypothesized from parabiosis (cross-circulation) studies that the *ob*/*ob* mouse lacked a circulating factor that contributed to this obesity, while the *db*/*db* mouse had this circulating factor but failed to respond

to it (Coleman 1973). During this time it was also shown that rats with lesions to the ventromedial hypothalamus produce phenotypes similar to those seen in the *ob*/*ob* and *db*/*db* mice, with parabiosis studies showing similar results to the *db*/*db* mouse (Hervey 1959). This suggested that the circulating factor acted in the hypothalamus to control appetite. Similar phenotypes were seen in human with trauma or disease to the hypothalamus, suggesting that similar energy regulating mechanisms were at work (Bray and Gallagher 1975). Finally in 1994 the specific gene defect in the *ob*/*ob* mouse was located, allowing the identification of the circulating factor as what is now called leptin, named for the greek word *leptós*, meaning thin (Zhang et al. 1994). The leptin receptors were identified shortly after in 1995 (Tartaglia 1995).

It was discovered that the *ob*/*ob* mouse lacked a functional form of leptin, while the *db*/*db* mouse lacked OB-Rb receptors for leptin. The obesity in these mice resulted from a marked increase in appetite and low energy expenditure. They also displayed shortness, infertility, and high circulating levels of glucose, insulin, and cortisol. However, all of these characteristics diminish in the *ob*/*ob* mouse with the administration of leptin (O'Rahilly 1998). In these mice leptin administration was found to induce weight loss through increased satiety and increased energy expenditure through heat production. After a month of leptin therapy these mice lost roughly half their weight (Halaas et al. 1995, Pelleymounter et al. 1995, Rentsch et al. 1995). Immediately recognizing the significance, Amgen bought the commercial rights to the hormone for $20 million in 1995, only six months after its discovery (Chicurel 2000). Leptin was quickly brought to the forefront of nutrition research with hopes of curing obesity in humans.

Physiology and Function:

Basic Physiology:

Leptin is 16 kDa protein made up of 167 amino acids, including a 21 amino acid terminal secretory sequence, thus circulating as a 146 amino acid protein (Caro and Considine 2004). It has a tertiary structure that places it in the cytokine family. It shows a high degree of homology among species, with human leptin being 84% identical to the mouse and 83% identical to the rat (Ahima and Flier 2000). In humans leptin is produced primarily in white adipose tissue. Leptin production is related to the level of adipose tissue, with those individuals having higher fat mass tending to produce more leptin (Klein et al. 1996). This is most likely due to increased leptin synthesis in larger adipocyte cells, as indicated by elevated levels of Leptin mRNA in such cells (Skurk et al. 2007, Bell and Considine 2006, Hamilton 1995). Subcutaneous fat stores produce more leptin then visceral fat (Hube et al. 1996, Montague et al. 1997). Leptin has a half life of 24.9 ± 4.4 min, with clearance occurring mainly in the kidneys (Jensen et al. 1999, Klein et al. 1996). Clearance rates are similar in males and females and either have no relation to adiposity (Klein et al. 1996) or being are decreased with increasing adiposity (Wong et al. 2004). Other sites of leptin production include brown adipose tissue (Kutoh et al. 1998), the ovaries (Loffler et al. 2001), the placenta (Masuzaki et al. 1997), mammary epithelium (Casabiell et al. 1997), and the stomach (Sobhani et al. 2002, Bado et al. 1998).

Leptin receptors are members of the cytokine receptor superfamily and have been found in a variety of sites, including the hypothalamus, liver, heart, kidneys, lungs, small intestines, pituitary cells, testes, ovaries, spleen, pancreas, adrenal glands, and adipose tissue (Margetic et al. 2002). The primary receptor site is in the hypothalamus, where leptin has been thought to act

upon the body's energetic regulation (Bates and Myers 2003, Veniant and LeBel 2003). There are at least six leptin receptor isoforms, falling into three goups: the short forms (OB-Ra,c,d,f), the long form (OB-Rb), and the soluble form (OB-Re) (Kutoh et al. 1998). Ob-Rd has been described only in rats while Ob-Rf has only been described in mice (Schulz and Widmaier 2006, Margetic et al. 2002). The reason for all of the various isoforms is as of yet not entirely clear and for the most part have been relatively understudied. It has been suggested that OB-Ra may be involved in leptin transport across the blood brain barrier (Kastin et al. 1999). Ob-Rc and Ob-Rd may function as a part of leptin clearance from the blood, while Ob-Re may act as a binding protein or stabilizing mechanism to regulate the bioavailability of leptin (Margetic et al. 2002, Huang et al. 2001, Lammert et al. 2001, Lee et al. 1996). OB-Rb is the most conserved across species, fully capable of activating intracellular signaling, highly expressed in the hypothalamus, and thought to be the most critical for energy regulation (Myers 2004).

All leptin signaling through OB-Rb relies on the initial activation of Janus kinase-2 (JAK2) (Villanueva and Myers 2008). This stimulates further events that lead to the activation of various signaling pathways, the primary one being the STATs (signal transducers and activators of transcription) pathways and others including the MAPK (mitogen-activated protein kinase) and PI3K (phosphoinositol 3-kinase) pathways (Lavens et al. 2006). Evidence from mice suppressing only the STAT3 pathway showed a phenotype w/ severe obesity but retained reproductive ability, suggesting leptin's pleiotropic affects are modulated through multiple pathways (Bates and Myers 2003). The downstream effects these pathways have on energy metabolism are not entirely known and appear to involve some level of redundancy; they include the inhibition of multiple orexigenic (appetite stimulating) peptides and stimulation of multiple

anorexigenic peptides (appetite suppressing). These orexigenic peptides include neuropeptide Y, melanin concentrating hormone, agouti-related protetin, galanin, orexin, and galanin-like peptide (Klok et al. 2007, Harrold 2004). The anorexigenic peptides include pro-opiomelanocortin, cocain- and amphetamine-regulating transcript, neurotensin, cotricotropin-releasing hormone, and brain-derived neurotrophic factor (Klok et al. 2007, Harrold 2004).

Anti-obesity role:

The hopes for leptin's role as an anti-obesity hormone in humans were short lived. Clinical trials failed to show the same results that were previously scene in mice (Chicurel 2000). The type of direct genetically controlled obesity *(ob/ob* or *db/db)* that was seen in mice turned out to be relatively rare in humans. Of the thousands of articles written specifically on leptin, only a handful have reported the identification of patients with genetic disorders similar to the *ob/ob* or *db/db* mice (O'Rahilly 2002, O'Rahilly 1998, Clement et al. 1998, Strobel et al. 1998, Montague et al. 1997). These case studies have shown that in these rare instances of leptin deficiency the same type of extreme obesity and most of the related phenotypic disturbances observed in mice can be seen in humans. In these cases it has also been demonstrated that treatment with leptin is an effective means of reversing many if not all of the associated symptoms of leptin deficiency, particularly obesity (O'Rahilly 2002). Although these case studies provide insight for studying the biology of leptin, the rarity of the genetic conditions has limited the prospect of leptin becoming the anti-obesity "wonder drug" as originally hoped. The clinical trials in humans without rare *ob/ob* homologies have showed variable responses (Phillips 1998) with results either failing to increase weight loss (Zelissen et al. 2005, Huckshorn et al. 2000), doing so at only very high doses of leptin (Heymsfield et al. 1999) or only when

combined with severe energy restriction (Huckshorn et al. 2003). This therefore begs the question, what is the physiological role of leptin in humans?

Thrifty Role:

Despite the many advances in leptin research, questions remain regarding the function of leptin in humans. The initial research in mice suggested that it plays a part in helping the body to regulate energetic status, acting as a homeostatic mechanism to regulate energy status in response to positive or negative energy balance (see Figure 2.1). Under this view leptin was seen as a mechanism to control obesity by causing increases in satiety and energy expenditure. More recent research suggests that leptin's main function is as part of a "thrifty genotype" designed to conserve resources in response to starvation (Flier 1998, Ahima et al. 1996). Evolutionarily the latter hypothesis makes sense in that humans have spent the majority of their evolutionary history in a state of limited and variable energy availability with low risk of becoming obese. Under this model the human energetic system can be seen as "hedging its bets" and is geared towards weight gain rather than weight loss or maintenance, due to the potentially high cost of weight loss (Schwartz et al. 2003). It should be kept in mind that during most of our evolutionary past weight gain would be comparatively small and may have been a short term or seasonal phenomenon, thus the risk of considerable weight gain and the cost involved were most likely negligible (Prentice et al. 2002). Therefore, obesity and the high leptin levels associated with it represent what could be considered a relatively novel state.

From an evolutionary perspective it makes sense that an organism should be sensitive to its environmental quality, particularly that which would affect the availability of energy. In

circumstances of limited energy availability it would be adaptive for an organism to be able to change how it allocates energy, for instance by investing less in reproduction and more in maintenance. Thus, many of these phenotypic traits that were originally associated with the obese *ob*/*ob* mouse have been identified as being similar to those that develop in response to starvation in many species, including humans (Friedman 2009). Following this logic, leptin has been hypothesized to function as an energetic signal to certain aspects of human physiology, including reproduction (Chehab et al. 2002, Laird et al. 2001), timing of the onset of puberty (Palmert et al. 1998), and immune function (Lord 2002).

An important point to be made here is that leptin, in all of these circumstances of adaptive physiological functioning, would be expected to be operating at comparatively lower levels of energy availability and at higher levels of expenditure then currently seen in modernized populations. Therefore, there is a need to determine what can be called an adaptive function and what may be better characterized as a mismatch between our evolved biology and current environment. This is important to keep in mind, in that most of what we know about leptin in humans comes from relatively energetically well off populations. Some evidence even suggests that at low leptin levels the relationship between fatness and leptin breaks down (Freeman et al. 2002, Eckert et al. 1998). However these studies may be flawed in that the first one is limited by the sensitivity of their leptin assay and they both use BMI as their marker of body composition, failing to control for differences in muscle wasting which may have led to or exaggerated differences in BMI, rather than actual differences in fatness. Clearly, in order to get a better understanding of the function of leptin, more information must be collected from

populations who show levels of adiposity and energetic stressors that resemble those experienced for the majority of human history (Prentice et al. 2002).

Leptin Resistance

Leptin's ability to induce increased satiety and weight loss at low doses in *ob*/*ob* individuals (Farooqi et al. 1999) has brought about the question of why it does not act in a similar fashion in genetically normal mice and humans (El-Haschimi and Lehnert 2003). As previously mentioned, leptin clinical trials in genetically normal obese human subjects fail to lose weight (Zelissen 2005, Huckshorn et al. 2000) or do so at only very high dosages (Heymsfield et al. 1999, see Mantzoros and Flier 2000 for a discussion). This has lead to the hypothesis that overweight and obesity result in a state of leptin resistance, where the satiety inducing effects of leptin are reduced at higher levels. This hypothesis is supported by overfeeding experiments, where the magnitude leptin rose above baseline was related to weight gain and fails to provide the expected anorexigenic effects (Kolaczynski et al. 1996a).

Two hypotheses have been proposed to explain why the high levels of leptin associated with the relatively novel condition of obesity do not induce weight loss. The first hypothesis is that obesity is due to a condition of leptin resistance, an idea similar to that of insulin resistance in diabetes (Friedman 2002). Under this hypothesis the development of tissue insensitivity to leptin is thought to limit the body's ability to utilize leptin's weight loss properties, thus causing obesity to occur over time (Munzberg et al. 2004). In much of the literature this hypothesis is cited as an explanation for why leptin treatment in cases of obesity fails to provide a significant effect. It is supported by data from diet-induced obese rats that show an attenuated response to

leptin injected into the lateral cerebroventricle compared to rats on a normal laboratory diet (Widdowson et al. 1997). One proposed mechanism for leptin resistance under this hypothesis is through a negative feedback loop. The most studied inhibitory molecule that have been implicated in such a feedback loop is SOC3 (suppressor of cytokine signaling) (Munzberg and Myers 2005). SOC3 expression is induced by the leptin receptor initiated JAK2/STAT3 pathway, thus levels increase in response to rising leptin levels. Rodent models lacking SOC3 have shown greater sensitivity to leptin and a lean phenotype (Howard et al. 2004, Mori et al. 2004). Additionally, in vitro studies have shown SOC3 to interfere with leptin receptor signaling by binding to JAK2 and an additional tyrosine residue (TYR_{985}) on the intracellular leptin receptor tail (Myers et al. 2008, Bjorbaek et al. 2000). Thus, as leptin levels increase, SOC3 increases in response and attenuates the effects of the increasing leptin.

The second hypothesis is that leptin acts in a nonlinear fashion due to a saturation effect. In this hypothesis leptin levels continue to increase in response to increasing adiposity but its ability to act as a satiety factor attenuated at higher levels. It has been suggested that transportation across the blood brain barrier may be a limited factor and that increased leptin past a certain point fails to reach the hypothalamus. This is supported by a decreased ratio of cerebral spinal fluid leptin to serum leptin with increased obesity (Caro et al. 1996a). Both of these hypotheses can be used to explain leptin's failure to treat obesity. More research will be necessary to clarify which hypothesis is the more likely explanation. However, regardless of which hypothesis is correct, they both suggest that leptin was not designed to manage the high levels of adiposity seen in many contemporary populations.

It has been suggested that in at least some species and under some conditions (i.e. hibernation, and pregnancy) leptin resistance may also offer adaptive benefits (Myers et al. 2008, Grattan et al. 2007, Chehab et al. 2004). Under these conditions gaining fat is beneficial, thus having a blunted response to rising leptin levels would be advantageous. In humans, pregnancy offers the most likely example, with leptin levels increasing significantly in the first trimester and then being followed by the increases in body fat that occur later in pregnancy (Highman et al. 1998). Leptin resistance during pregnancy may be accomplished by some of the increased amounts of the leptin being sequestered by higher levels of the soluble leptin receptor (Henson and Castracane 2006, Huang et al. 2001). Leptin resistance may also occur only in select tissues, with studies in mice suggesting that resistance may be occurring in the hypothalamus, while leptin receptors in the heart and other peripheral sites still respond to the increasing leptin levels (Tschop et al. 2007, Somoza et al. 2007, Correia et al. 2002). This may be relevant during human pregnancy where the resistance may be occurring at the hypothalamus while the increased levels of leptin serve additional functions, such as involvement in embryonic implantation or fetal growth and development (Henson and Castracane 2006). Additionally it may explain some of the negative health consequences of obesity that have been associated with increased leptin levels as a causal agent, such as hypertension (Mark et al. 2002).

The concept of leptin resistance has been met with criticism from some researchers. The use of the term leptin resistance and its relationship to obesity has been criticized due to the fact that high leptin level's association with obesity doesn't necessarily make it the main causal factor in obesity nor is it even necessarily one of leptin driven dysfunction, that the term may be incorrectly pathologizing leptin's role in obesity (Prentice et al. 2002, Arch et al. 1998). It has

also been argued that the conclusion of "leptin resistance" is based on the false assumption that a homeostatic adiposity set point is set low in all individuals, while in reality the system is working perfectly, with obesity resulting from high adiposity set points in some individuals that have only surfaced in the modern environment (Speakman 2006).

Leptin – Appetite and Satiety effects

Besides obesity, one of the major phenotypic characteristics of genetically leptin deficient humans is that they are hyperphagic (Farooqi et al. 2009, Monatgue et al. 1997). This has led to a lot of interest in leptin's role in satiety and eating behavior. However, due to the fact that leptin does not respond to meals before 4 hours (see *Dietary Composition* section) its role in short term satiety is limited (de Graaf et al. 2004). In a state of neutral energy balance leptin appears to not be related to measures of hunger and satiety (Romon et al. 1999, Joannic et al. 1998, Karhunen et al. 1997). However, when in negative energy balance measures of hunger (Mars et al. 2006, Doucet 2004, Keim et al. 1998, Heini et al. 1998), satiety (Heini et al. 1998), and actual intake (Chin-Chance et al. 2000) are associated with leptin levels. However, this relation of appetite to actual behavior is unclear, as in other studies the magnitude of leptin decrease was not predictive of ad libitum calorie consumption following a fast or underfeeding in men (Cooper et al. 2009, Mars et al. 2005). Administration of exogenous leptin has also been seen to either decrease appetite during negative energy balance (Schurgin et al. 2004, Huckshorn et al. 2003, Westerterp-Plantenga et al. 2001) or have no not effect at all (Chan et al. 2003).

To date, experiments in overfeeding have failed to measure appetite and satiety, so the effects of positive energy balance are unknown. However, actual food intake has been measured.

Chin-Chance et al. (2000) found intake decreased with increased leptin. There was a similar, although non-significant pattern seen by Cooper et al (2009). However, in another study intake was seen to be increased with overconsumption, despite higher leptin levels (Jebb et al. 2006). These studies may be indicative of a weakened leptin effect during overconsumption or various levels of leptin resistance in the particular study subjects.

Thus, the evidence suggests that leptin does act to increase appetite, but usually during negative energy balance, following its role as a thrifty, energy conservative signal.

Leptin – Regulator of other functions

Leptin's main role can be seen as a metabolic switch, acting to provide adaptive physiological changes during times of decreased energy availability (Himms-Hagen 1999). Under this role it acts to regulate the complex endocrine responses to negative energy balance and provides a clear signal of deficiency (Flier 1998). Administration of leptin to mice and humans in the fasted state acts to blunt at least some of the responses in the HPG and HPT axis normally associated with negative energy balance, providing further support for leptin's role in this capacity (Chan 2003, see Table 2.1a and 2.1b for examples). This section will provide a brief review of leptin's main proposed pleiotropic effects, including those involving reproduction, immunity/inflammation, and bone density.

One of the main systems that have been suggested to be influenced by leptin is the reproductive system. This connection was made in the original 1950 article of the OB leptin deficient mice, whose characteristics included sterility (Ingalls et al. 1950). Shortly after leptin was identified, recombinant leptin was shown to reverse this sterility (Chehab et al. 1996).

Leptin receptor deficient human females have phenotypes with hypogonadism, delayed puberty, and irregular menses after the age of 20, all suggesting leptin plays an important role in human reproductive function (Farooqi et al. 2007). It is seen as being important to various aspects of reproduction, including puberty (Kaplowitz et al. 2008), fecundity (Demir et al. 2007), and pregnancy (Henson and Castracane 2006). The strongest evidence for leptin's connection to reproduction is that low leptin levels accompany exercise induced amenorrhea in humans (Laughlin et al. 1997). Treatment with recombinant leptin has been shown to reverse the exercise-induced amenorrhea (Welt et al. 2004). However, current evidence suggests that leptin does not play a role in the length of lactational amenorrhea (Tennekoon et al. 2005).

Part of leptin's role in providing a signal of insufficiency may be in down regulation of immune function, providing an important link between malnutrition and susceptibility to infection (Flier 1998). Early research in humans showed that leptin receptors can be found on $CD4^+$ and $CD8^+$ T lymphocytes, as well as on T_{reg} cells, and *in vitro* research shows that leptin enhances lymphocytes stimulation, providing evidence that leptin may act directly to impact immune function (DeRosa et al. 2007, Martin-Romero et al. 2000, Lord et al. 1998). Absence of leptin from *in vitro* samples reduces T cell proliferation, with the subsequent addition of leptin restoring it (Chan et al. 2006). Authors of this study suggested leptin at a certain threshold level may be necessary to maintain proper immune function (Chan et al. 2006).

Another *in vitro* study showed that monocytes express leptin receptors and leptin acts to stimulate increased proliferation and increased expression of monocyte activation markers (Zarkesh-Esfahani et al. 2001, Santos-Alvarez et al. 1999). Data from a Turkish pedigree known for *ob* deficiency showed a high degree of child mortality in those children presenting an obese

phenotype (7 out of 11) compared to those presenting a normal weight phenotype (0 out of 19), with researchers suggesting the increased mortality rate may be tied to leptin deficient immunosuppression (Ozata et al. 1999). Children with confirmed leptin deficient genotype have shown higher rates of childhood infections compared to their normal genotype siblings (Farooqi et al. 2002). These children also showed decreased $CD4^+$ T cells, as well as decreased proliferative lymphocyte responses and lower production of cytokines to a variety of stimuli, all of which was normalized with leptin treatment. Overall this evidence does suggest leptin has some involvement with the immune system.

In conditions of energy surpluses, with high leptin levels may help to explain increased rates of autoimmune diseases or CVD related inflammation (Matarese et al. 2002). Data from patients with a variety of immune-mediated inflammatory as well as autoimmune diseases have shown mixed results, suggesting a complex relationship with leptin (Lam 2007, Bernotiene et al. 2006). The system relief experienced by fasting during some autoimmune diseases has been suggested to be leptin mediated (Kuchroo and Nicholson 2003). Experiments with leptin administration do not increase cytokines or inflammatory markers in short term 3 day fasted subjects (Chan et al. 2005). However, long term (~5 years) amenorrheic women did show increased levels of the soluble TNFα receptor levels (Chan et al. 2005b). Any relationship between these variables may simply be due to the confounding effects of obesity, rather than true causality (Matarese et al. 2005). Alternatively, the relationship between leptin and immune function may be very complex, with leptin playing a role as one of many involved adipocyte derived hormones (Maclaren et al. 2007).

It has been suggested that leptin has two opposing roles in bone mineral density (BMD), one where it acts through the central nervous system to inhibit bone formation via the sympathetic system and another where it directly stimulates bone formation peripherally (Kawai et al. 2009, Rosen et al. 2006). In humans, BMD has been shown to increase with adiposity and decrease with low leptin levels, seen in amenorrheic athletes (Kaufman et al. 2002) or anorexia nervosa patients (Mueller et al. 2008). It has also been shown to decrease in those that experience weight loss (Shapses and Riedt 2006). These associations suggest that the direct action of leptin is more important or that a state of leptin resistance during obesity may act to blunt the sympathetic system response. Receptors for leptin have been found on osteoblasts and an *in vitro* study suggests that leptin does indeed stimulate bone mineralization in humans (Reseland et al. 2001b). Various studies have attempted to examine the relationship between leptin and bone mass, but have resulted in mixed results and can largely be considered inconclusive, with studies showing positive (Blain et al. 2002, Yamauchi et al. 2001) or no association (Filip and Raszewski 2009, Jurimae and Jurimae 2006, Odabasi et al. 2001, Martini et al. 2001, Goulding and Taylor 1998, Rauch et al. 1998). Two studies showed a positive association with leptin and BMD in women, but no association in men, suggesting the relationship may be sex dependant (Weiss et al. 2004, Thomas et al. 2001). Four studies also showed a negative association in men (Lorentzon et al. 2006, Morberg et al. 2003, Sato et al. 2001), with one also showing it in premenopausal women (Chanprasertyothin et al. 2005). Most of the positive correlations were seen in post-menopausal women. Administration of recombinant leptin to amenorrheic athletes showed a positive effect on markers of bone formation (Welt et al. 2004). Although varied these results suggest that the relationship between leptin and bone may only exists in more high

risk groups (Postmenopausal women, anorexics, etc) (Arabi 2005) or alternatively it has been suggested that its primary relationship with bone may occur during times of growth, such as puberty (Hamrick 2004). However, genetically leptin deficient children show normal growth (no stunting) and age appropriate bone mineral count and density (Farooqi et al. 2002).

Although not extensively reviewed here, leptin has been implicated as a causal or contributing factor in a number of disease states beyond obesity, some of which are related to leptin's relationship to inflammation. These include various cancers (Alexe and Petridou 2006, Garofalo and Surmacz 2005, Markowska et al. 2004), cardiovascular disease (Guallo 2007, Luo et al. 2005, Wallace et al. 2001), hypertension (Haynes 2005), and anorexia nervosa (Muller et al. 2008, Mantzoros et al. 1997).

Causes of Variation:

Diurnal Variation:

Twenty-four hour leptin profiles show that diurnal variation exists. There is an increase in evening levels with a peak occurring between one and four a.m. (Licinio et al. 1998, Himms-Hagen 1999) while levels are lowest around noon to mid afternoon (Sinha 1996). Changing the timing of meals by 6.5 hours while maintaining light and sleep patterns results in the diurnal rhythm correspondingly shifting by five to seven hours (Schoeller 1997). This suggests that the diurnal pattern is tied to meal timing rather than true circadian regulation. Sinha and Caro have suggested that the night time rise may be related to cumulative hyperinsulinemia from food ingestion throughout the day (Singha and Caro 1998). Lack of sleep and decreased sleep (4 hours) have also been shown to have an effect, decreasing the amplitude of the night time rise

(Mullington et al. 2003), as well as total 24 hour mean levels (Spiegel et al. 2004, 2005). This could result from increased sympathetic nervous system activity, which inhibits leptin (Spiegel et al. 2005). Obese individuals have been found to exhibit a blunted diurnal pattern compared to lean subjects (Perfetto et al. 2004, Saad et al. 1998). Females also have a blunted diurnal pattern compared to males, the significance of which is unclear, but may simply be a function of their increased body fat (Saad et al. 1998). The diurnal rhythm has been reported to be absent or reduced in amenorrheic athletes (Laughlin and Yen 1997), in underweight women with anorexia nervosa (Balligand et al. 1998, Stoving et al. 1998), and in fasting individuals (Boden et al. 1996). Currently, it is not entirely clear what measure of leptin, (peak, nadir, overall level, etc) is most important in terms of physiological function and the most appropriate to measure, with the majority of studies utilizing fasting morning values, more for consistency and ease, rather than theoretical importance.

It has been suggested that leptin has a pulsatile pattern. Detecting this pattern is somewhat problematic, with short pulsatile times resulting in loss of resolution with increased time between sample collections. Seven minute sampling for twenty four hours has detected pulse durations of 32 ± 1.6 minutes and an average interpeak interval of 43.8 ± 1.8 minutes (Licinio et al. 1997). Pulsatility is not related to measures of fatness (Koutkia et al. 2003). A comparison between one lean and one obese subject showed the increased mean 24 hour leptin levels in the obese individual resulted from increased pulse height, with otherwise similar pulse characteristics (Licinio et al. 1997). Some researchers have noted that the pulsatile pattern is impacted by and may even result from random errors and variation in sampling or limitations of the assay (Ribeiro et al. 2006, James et al. 2002). It should be kept in mind that the research on

both diurnal and pulsatile patterns comes from a limited number of studies, with little understanding of the possible range of variation seen in humans.

Sexual Dimorphism in Leptin:

Women have been observed to have higher levels of leptin then men, with levels typically being two to three times higher in women (Wauters and Van Gaal 1999). One explanation for this is that females have a higher amount of body fat compared to males. Attempting to evaluate this explanation has been difficult given a number of methodological problems in the research, including lack of standardized collection methods and analysis.

A number of studies have attempted to examine the difference between the sexes by comparing leptin using BMI as an indicator of fatness (Al-Harithy 2004, Ma et al. 1996). This is problematic due to the differences in the relationships between BMI and fatness between men and women and the fact that BMI is a weight-for-height index rather than a true measure of adiposity (Wells 2010, Jenkins and Campbell 2003, Hortobagyi et al. 1994, Deurenberg et al. 1991). This could potentially result in spurious sex differences, thus those studies relying solely upon BMI will be ignored.

Some studies that show sexual dimorphism (Sumner et al. 1997, Havel et al. 1996, Rosenbaum et al. 1996) have been criticized for their use of ratios to correct for fatness and compare across the sexes (Marshall et al. 2000). This may be misleading and act to create spurious differences given a non-zero y-intercept of the true regression between leptin and fatness or a nonlinear relationship as fatness increases, with regression modeling seen as a better alternative (Marshall et al. 2000, Jasienski and Bazzaz 1999, Rosenbaum and Leibel 1999).

Criticisms have also been made (by Jensen et al. 1999) against studies that fail to log transform skewed leptin data in their analysis (see Kennedy et al. 1997). Lastly there is disagreement about what is the most appropriate and physiologically important measure of fatness. Jensen argues that percent body fat is most appropriate in that it explains more of leptin's variation (in their study) and proportion of body fat should matter more than absolute values as it does in other biological systems, giving the example that "insulin resistance is more likely to be present in small individuals with a high percent body fat then large individuals with the same body fat mass, but a lower percent body fat" (1999: 244). However, considering leptin's direct relationship with adipocyte size discussed in the basic physiology section earlier, the comparison to insulin may be inaccurate. Furthermore, Rosenbaum (1996) argues that fat mass is a better predictor, due to percent fat being a relative measure on a fixed interval (0-100%). Thus, small changes in fat mass in slender individuals will result in large changes in percent body fat while large changes in fat mass in very obese individuals will result in very small changes in percent body fat (giving it a curvilinear relationship). This really comes down to the idea of which is more important absolute or relative levels of fatness, with the available data being divided on this issue. This question of what is the better of predictor of leptin is a difficult one to answer, with percent body fat (Marshall et al. 2000, Jensen et al. 1999) and fat mass (Bennet et al. 1997, Saad et al. 1997, Rosenbaum et al. 1997, Rosenbaum et al. 1996) each being the better predictor in different studies. Until a clear physiological rational can be made for one particular measure, it has been recommended to explore both (Marshall et al. 2000).

The question of whether a sex difference persists after controlling for fatness seems equally confusing. A number of studies have shown that when controlling for percentage body

fat the difference is indeed explained (Considine et al. 1996, Maffei et al. 1995), although not in many of the same studies when controlling for fat mass instead (Marshall et al. 2000, Jensen et al. 1999, Bennet et al. 1997). However, when Bennet et al. limited their analysis to individuals with BMI's less than 32.7 the sex differences did remain in models controlling for percentage body fat or fat mass (1997). Other studies have shown that the sex differences persist when controlling for percent body fat (Guerra et al. 2008, Staiger et al. 2003, Ostlund et al. 1996), fat mass (Marques-Vidal et al. 2009, Yannakoulia 2003, Saad et al. 1998, Rosenbaum et al. 1997, Couillard et al. 1997, Niskanen et al. 1997, Elbers et al. 1997), fat volume (Considine et al. 2008) or multiple measures of fatness (Saad et al. 1997).

A real sex difference may be at least partially explained by the different distribution of body fat in men and woman, with women having a higher level of subcutaneous fat compared to men, which as discussed earlier produces more leptin (Bennett 1997). However, when higher subcutaneous fat in woman are controlled for this difference still persists, at least in obese subjects (Iglesias et al. 2006, Wauters and van Gaal 1999). The physiological data tends to support the existence of real sex based differences. One *in vitro* study showed that women have an increased rate of leptin secretion per unit fat mass in subcutaneous fat (Hellstrom et al. 2000).

The reproductive hormones have been linked to leptin levels with *in vitro* studies showing estradiol stimulates secretion of leptin from visceral adipose tissue (Casabiell et al. 1998), while dihydrotestosterone inhibits it (Pineiro et al. 1999, Wabitsch et al. 1997). Further evidence, particularly for the effects of testosterone comes from transsexuals undergoing sex reassignment, where sex hormone milieu and adiposity were significant determinants of leptin levels after treatment, while genetic sex was not (Elbers et al. 1997). In that study female to male

transsexuals receiving treatment with testosterone experienced a marked decrease in leptin levels, while male to female transsexuals receiving estradiol and an anti-androgen showed increased levels. Treatment with testosterone has also been shown to lower elevated leptin in hypogonadal men (Jockenhovel et al. 1997). A number of other studies have shown a negative relationship between testosterone levels and leptin levels in males (Guerra et al. 2008 (didn't control for body fat), Carraro and Ruiz-Torres 2006, Luukkaa et al. 1998, Vettor et al. 1997, Wabitsch et al. 1997), while Kristensen et al failed to find one after controlling for fatmass (2000). In one study administration of estrogen in normally cycling women was shown to increase leptin levels (Lin et al. 2005), while in another study estrogen alone did nothing and a estrogen and progesterone combination lead to increased leptin (Messinis et al. 2001). However, estrogen replacement therapy following bilateral ovariectomy failed to increase leptin levels (Nar et al. 2009). Observational studies also show mixed results for the leptin and estrogen relationship, with it supported in some studies (Hong et al. 2007, Puder et al. 2006, Unkila-Kallio et al. 2001), not detected in others (Alexander et al. 2009. Lambrinoudaki et al. 2003, Thong et al. 2000), or disappears after controlling for fatness (Castracane et al. 2006, Kristensen et al. 2000). A study by Martin et al supports the idea that testosterone, but not estrogen, combined with body composition differences explains the sex based differences in leptin (2002).

In summary, females have leptin levels 2 to 3 times higher than males. This is at least partially explained by body composition differences (amount and distribution of fat) and potentially by differences in reproductive hormones, particularly testosterone. The significance of this sex difference, if any, is as of yet unknown, although it has been suggested that this

difference may reflect leptin's involvement in reproduction in women (Wauters and Van Gaal 1999).

Energy Balance:

Leptin has been shown to be positively associated with adipocyte size in humans and when in neutral energy balance has often been thought to be secreted in proportion to levels of fatness (Skurk et al. 2007). Under conditions other then neutral energy balance leptin levels will change preceding any changes in body fatness. Fasting has been shown to lead to decreased levels, with re-feeding leading to a reversal of this effect (Cooper et al. 2009, Trayhurn and Beattie 2001, Bergendahl et al. 1999, Grinspoon et al. 1997, Weigle et al. 1997, Pratley et al. 1997, Boden et al. 1996). This decrease in levels is in excess than what would be expected based solely upon fat loss, suggesting a true response to the condition of negative energy balance (Chan et al. 2003). The decreasing effect of fasting has been reported to occur between 12 (Kolaczynski et al. 1996) and 16 hours (Korbonits et al. 1997) after the start of a fasting period, with levels returning to baseline within 12 hours of refeeding (Weigle et al. 1997). Fasting may cause an increase in leptin clearance in addition to a decrease in leptin production and these effects may be blunted in obese individuals (Chan et al. 2008, Klein et al. 2000). This suggests that leaner individuals may be more sensitive to the effects of negative energy balance. Restricted energy diets also shown decreases in leptin beyond that expected by body fat changes (Mars et al. 2006, Doucet et al. 2004, Wolfe et al. 2004, Wadden et al. 1998, Keim et al. 1998, Considine et al. 1996). Eating one meal a day compared to three meals showed no effect on morning fasting leptin levels (Carlson et al. 2007). History of weight cycling doesn't seem to

impact leptin, as overweight or obese women who underwent greater periods of repeated weight loss and regain are not associated with changes in leptin levels compared to those that tend to maintain their weight (Strychar et al. 2009). Baseline levels and changes in leptin following weight loss also fail to predict success at maintaining a lower weight (Wing et al. 1996).

This decrease in leptin levels seen with negative energy balance during fasting or weight loss has been suggested to act as a signal to other physiological systems, indicating the need to conserve energy (see *Thrifty Role* above). This is supported by experiments where during periods of fasting or weight loss leptin is administered in an effort to counter act the negative energy balance associated decrease. Although extremely detailed, it should be kept in mind that all of these experiments have relatively small sample sizes ($n < 10$ for all studies). Some of the most recent findings show that weight loss has been reported to change how the brain responds to visual food stimuli, while supplementing leptin levels reverses this effect (Rosenbaum et al. 2008, Ahima 2008). Table 2.1 provides a summary of some weight loss associated biomarkers, with leptin restoring some, but not all, of these physiological changes associated with energy deficiency. These data clearly show leptin's important regulatory role in times of energy deficits.

Overfeeding results in increased leptin secretion with levels that are higher than expected based on gains in body fat. Evidence suggests the response may be less dramatic than that seen in underfeeding (Hagobian et al. 2008, Chin-Chance 2000, Ohannesian et al. 1999, Kolaczynski et al. 1996). However a recent study in obese males showed that leptin levels did not increase with overfeeding, except when an individual had previously been exposed to a period of underfeeding (Cooper et al. 2009). This may indicate that obese individuals have a reduced hormonal

response to positive energy balance and thus are more prone to overfeeding. Unlike the effects of underfeeding upon non-resting energy expenditure (see Table 2.1), overfeeding may not impact energy expenditure, with evidence showing no change in non-exercise activity thermogenesis (Levine et al. 1999).

In summary, a state of negative energy balance results in a decrease in leptin levels that is associated with other energy saving physiological responses. Many of these responses can be reversed if leptin levels are artificially raised. A similar, potentially lesser, response is also typically seen in the presence of positive energy balance.

Resting and Total Energy Expenditure

Several studies have shown no relationship between RMR or BMR and leptin after controlling for fat free mass (Usui et al. 2007, Johnstone et al. 2005, Nies et al. 2004, Maguire et al. 2002, Verdich et al. 2000, Filozof et al. 2000, Soares et al. 2000, Kennedy et al. 1997, Niskanen et al. 1997, Roberts et al. 1997, also see Table 2.1b for no effect from leptin administration). In a few studies there has been reported a relationship between RMR and leptin (Jeon et al. 2003, Jorgensen et al. 1998, Nicklas et al. 1997). More conclusively however, in a carefully controlled study in non-obese males on a balanced energy diet researchers found no changes in RMR following treatment with leptin (Mackintosh and Hirsch 2001). Additionally, genetically leptin deficient individuals show no acute or chronic changes in BMR or total energy expenditure (controlling for lean body mass) after treatment with leptin (Farooqi et al. 2002, Farooqi et al. 1999).

Weight loss of 10% fails to decrease resting energy expenditure (REE), but total energy expenditure (TEE) is decreased, largely as a result of decreased non-resting energy expenditure (NREE) (due to increased muscle efficiency). These changes are not associated with leptin levels after controlling for fat mass and fat free mass (Rosenbaum et al. 1997). However, when administered leptin, individuals show an increase in TEE and NREE (corrected for body size) back to pre-weight loss values (Rosenbaum et al. 2002). This suggests that leptin may indeed play a role in their decrease, however that role may be either nonlinear, some kind of threshold effect, or may interact with other factors.

A carbohydrate overfeeding experiment showed similar results, with leptin levels increasing, no change in BMR or physical activity related energy expenditure, but an increase in TEE (Dirlewanger et al. 2000). The increased TEE was most likely from non-exercise physical activity and was not related to the increase in leptin levels.

Overall, unlike rodents, most evidence suggests leptin is not directly related to resting energy expenditure in humans (Hukshorn and Saris 2004). However it may play a role in the decrease in total energy expenditure associated with weight loss.

Physical Activity

The relationship between physical activity and leptin levels is a very complex one with various studies often reporting conflicting results. Part of this disagreement stems from the different methodologies, with a large range of measures constituting physical activity and associated variables, including intensity, volume, duration, and subject's initial and final level of conditioning (Fatouros et al. 2005). Various studies can also be criticized for their lack of

control for energy balance (e.g. Essig et al. 2000 or Gomez-merino et al. 2002) or adiposity (e.g. O'leary et al. 2005) in attempting to study the effects of physical activity. Methodological criticisms also include measurement of only a single morning fasting sample (Hickey and Calsbeek 2001) or failing to control for circadian rhythms or hemoconcentration (Kraemer et al. 2002). It should also be kept in mind that the majority of these studies have focused upon male participants, with relatively less work being done with females. Generally these studies on physical activity can be put into three groups: cross-sectional studies, short term or single bout studies, and longer exercise training studies (Hickey and Calsbeek 2001).

Cross sectional studies typically reveal that athletes have lower levels of leptin that is associated with their low measures of body fat. This is seen in a comparison ranging from marathon runners to bodybuilders and sedentary controls (Gippini et al. 1999, Leal-Cerro et al. 1998). A study comparing professional football players to sedentary controls showed lower leptin levels in the athletes, but failed to control for their lower body fat percentage (Unal et al. 2005). One study showed no difference in fasting leptin levels when comparing male sedentary controls, joggers, and weight lifters who all had similar levels of fat mass and body fat percentages (Tsao et al. 2007). Tomaszewski et al. (2003) found that leptin was lower in ultramarathon runners compared to sedentary controls in both lean and nonlean groups. However groups were made using a BMI cutoff, with no other variables used to control for adiposity. The majority of cross-sectional studies show that the lower levels of body fat are most likely the cause of the lower leptin levels in more physically active people.

Short term studies have tended to show that activity has no effect upon fasting morning (Houmard et al. 2000, Dirlewanger et al. 1999), during exercise (Zoladz et al. 2005, Racette et al.

1997), post-exercise leptin levels (Hagobian et al. 2008, Kyriazis et al. 2007, Jurimae et al. 2005, Zafeiridis et al. 2003, Torjman et al. 1999, Perusse et al. 1997, Hickey et al. 1996) or across multiple collection points (Sartorio et al. 2004, Weltman et al. 2000, Landt et al. 1997). Additionally, seven consecutive days of exercise for 60 min/d at 75% maximal oxygen consumption had no effect upon fasting morning leptin levels (Houmard et al. 2000). An eight day intense training camp did not change morning leptin levels in elite endurance runners (Ishigaki et al. 2005). A four day treadmill exercising protocol (resulting in an expenditure of 30 kcal/kg LBM/day) showed that any impacts upon 24 hour leptin mean and amplitude in female participants resulted from the energy costs, not physical activity independently (Hilton and Loucks 2000). A study of individuals running a 42 km marathon and eating before, during, and after the marathon showed no difference in levels one hour after the race (Koistinen et al. 1998). However, in the same study individuals showed some decreases in leptin while exercising in a fasted state for 3 hours on a cycle ergometer.

Some short term studies did reveal decreases in leptin levels following intense exercise sessions in trained subjects. Leptin decrease post exercise following maximal voluntary exhaustion after 30 minutes at rest in college level male rowers, however two of the eight rowers measured may be responsible for the significance (Jurimae et al. 2006b). Another study among eight elite level rowers showed decreased levels immediately following a progressive exercise test to exhaustion and 30 minutes after (Jurimae et al. 2007). Legakis et al (2004) showed levels decrease after 20 minutes of exercise in middle age male and female subjects, but return to pre-exercise levels after an hour at rest. All three of these studies had small sample sizes (n = 8 to 12) and failed to use non-exercise controls. A small, but significant reduction in leptin was seen in

trained runners following a 42.196 km marathon, with an estimated average energy expenditure of 2800 calories (Leal-Cerro et al. 1998). Elias et al (2000) showed that after a 12 hour fast leptin levels following a treadmill test to exhaustion resulted in decreased leptin levels 30 to 120 minutes following exercise, however they failed to include a control group. A study by Nindl et al (2002) showed that following an acute heavy resistance training protocol (estimated energy expenditure = 856 kcal) leptin levels were shown to be decreased after 9 hours. A 25 km swim race resulted in decreased post exercise leptin levels in trained athletes (Karamouzis et al. 2002). Desgorces et al (2004) showed decreased leptin levels at 120 minutes and 24 hours following a 90 minute rowing session, however six months after they started training they only showed the 120 minute decrease, while at 24 hours their levels had returned to pre-exercise levels. However, the participants were experiencing negative energy balance at both time points, although less at the later point. One study showed that three hours of bicycling resulted in decreased leptin levels, but was counteracted if a 750 Kcal carbohydrate drink was ingested during exercise, suggesting the exercise effect is sensitive to energy availability or the presence of carbohydrates (Keller et al. 2005).

A study of ultramarathon runners completing a 101 mile race showed decreased levels immediately post-race with levels increasing 18-24 hours later (Landt et al. 1997). Duclos (1999) showed that after a two hour run and two hours at rest, trained runners showed decreased leptin levels. Olive and Miller (2001) showed that that in healthy, active males a short maximum intensity exercise session had no effect on leptin, while an hour long endurance session (expending ~900 kcal) decreased leptin at both 24 and 48 hours post-exercise. A study by Van Aggel-Leijssen et al (1999) showed that in males an exercise protocol (4 bouts for 30 minutes

each, ~ 800 kcal) occurring in neutral energy balance resulted in a reduction in the weighted average 24 hour and peak leptin levels. Interestingly Van Aggel-Leijssen et al's positive energy balance and exercise protocol showed an increase in the amplitude of the 24 hour leptin curve, perhaps providing evidence that energy balance can counteract any exercise related decreases. This is supported by another study that also showed overfeeding and exercise resulted in no change in leptin levels (Hagobian et al. 2008)

A single study showed that after accounting for exercise related changes in hemoconcentration leptin levels did not change during or immediately after exercise, but showed an increase that started 90 minutes post exercise (Fisher et al. 2001). It is not entirely clear why this study differed from all of the other studies conducted. Overall it appears that short term exercise has little effect on leptin levels under fasting or exercise conditions, and that longer, intense exercise sessions may result in decreased post-exercise levels, however many of the studies showing these post-exercise effects have methodological problems failing to control for energy balance or to have a control group.

Longer exercise training studies have also shown little changes in leptin. Moderate aerobic training program ranging from two to twelve months showed exercise had no independent effects upon fasting leptin levels (Hasbum et al. 2006, Desgorces et al. 2004, Ozcelik et al. 2004, Thong et al. 2000, Christensen et al. 1998, Perusse et al. 1997), except for one study with decreased levels in females (Hickey et al. 1997). However, this difference is most likely due to the effects of negative energy balance, as suggested by their unchanging dietary intake, despite the extra exercise burden. A one year resistance training program did show intensity related decreases in leptin, however this may have also been due the effects of weight

loss and negative energy balance (Fatouros et al. 2005). A year long endurance exercise study also showed decreased leptin levels, but was again seen under conditions of negative energy balance (Reeseland et al. 2001b). A nine week study with highly trained rowers showed decreased fasting leptin levels with high intensity resistance training (Simisch et al. 2002). Pasman et al showed that after 4 months of a low energy diet and endurance exercise training, in those individuals who continued only the endurance training showed decreased levels of leptin compared to those who dropped both conditions (1998). However, this study, even though it did control for body fat percentage, failed to control for the effects of the greater level of positive energy seen between the controls, as evidenced by their greater weight regain. Ramson et al showed that during a four week study with rowers an increase in training volume resulted in decreases in post workout (5 minute and 30 minute) leptin levels (2008). In a five week study on highly trained rowers, Jurimae et al (2003) showed that morning fasting leptin levels decreased during weeks with intense training compared to baseline or lighter weeks. Additionally, at the end of the heavy training session leptin levels decreased in response to a maximal rowing ergometer test, while the same test didn't result in decreased levels during weeks with lower training intensity. This study failed to measure energy balance and did not report changes to the athlete's weight or bodyfat over the course of training. Much like in short term studies, these long term studies largely show no effect or only an effect under very high levels of exercise stress, with many of the studies demonstrating an effect having similar methodological problems (i.e. failing to control for energy balance).

Dietary Composition:

Beyond the focus on dietary energy intake, there has also been interest in how dietary composition affects leptin. This work has primarily focused upon macronutrient profiles, mainly lipids and carbohydrates, with relatively less work focusing on proteins. Any effect is also thought to be delayed as early studies showed no acute leptin response three hours post meal (Korbonits et al. 1997). An isocaloric dietary fat reduction did not have an effect on leptin levels (Weigle et al. 1997), nor did diets ranging from 14% to 60% fat (Schrauwen et al. 1997, Havel et al. 1996). Individual meals of either high or low fat content following an overnight fast had no effect on leptin levels, nor did a seven week reduction in dietary fat from 37% to 10% (Weigle et al. 1997). A 24 hour lipid infusion resulted in no change in leptin levels (Kopecky et al. 2010). Overfeeding 40% total calories on fat for three days did not result in changes in the following morning fasted leptin levels, while overfeeding on carbohydrates increased levels (Dirlewanger et al. 2000, Dirlewanger et al. 2000). However, another study did show that five days of overfeeding with fat increased leptin levels (Brons et al. 2009). Consumption of omega three fatty acids supplements led to a non-significant decrease ($p = 0.053$) in leptin compared to those individuals consuming controls (Reseland et al. 2001).

Four to nine hours after ingestion of a carbohydrate meal compared to a fat one resulted in increased leptin levels, with obese subjects showing a lesser response (Romon et al. 2003, Romon et al. 1999). This may be a fixed time delayed effect, with another study showing a high carbohydrate meal after a 17.5 hour fast elicited no post meal effect up to four hours (Lopes et al. 2001). Another study confirmed this with a mixed macronutrient meal resulting in higher leptin levels compared to fasting after four to five hours (Dallongeville et al. 1998). Evidence suggests

that carbohydrates must be present for this effect, with pure fat intake, oral or intravenous, eliciting no response compared to a mixed meal (Evans et al. 2001). Women eating a high fat/low carb meal showed lower leptin levels over a 24 hour period compared to a low fat/ high carb meal (Havel et al. 1999). A low carbohydrate diet compared to a low fat diet results in a greater decrease in leptin levels (de luis et al. 2007). After four days of dieting, the fall in leptin has also been correlated with amount of decreased carbohydrate intake, but not protein or fat (Jenkins et al. 1997). There is some indication that type of carbohydrate consumed matters, with fructose consumption eliciting a 24 hour profile with a depressed nocturnal peak compared to glucose consumption (Stanhope et al. 2008, Teff et al. 2004). Fiber supplementation (20g) did not have any effect upon fasting leptin levels (Heini et al. 1998). Ingestion of amino acids did not have any effects upon leptin levels up to 300 minutes post-ingestion (Knerr et al. 2003, Groschl et al. 2003).

From this evidence it appears that dietary fat and protein have little effect upon leptin levels, while overfeeding carbohydrates may increase levels, with post meal effects delayed up to four hours. A decrease in carbohydrates may also have a decreasing effect on leptin levels. These changes may be mediated by insulin or glucose metabolism and will be discussed further below.

Leptin – Insulin and Glucose

Insulin has been considered by many to be a prime candidate for stimulating leptin's release, particularly in relation to nutritional factors (Ahima and Flier 2000). However, the data linking insulin to leptin has been mixed. Insulin resistant men show higher levels of leptin

compared to matched controls (Segal et al. 1996), however this was not the case in Pima Indians (Pratley et al. 1996). An *in vitro* study using human adipocyte cultures showed increased leptin production in the presence of insulin (Kolaczynski et al. 1996). Several studies using euglycemic clamps failed to show a response to insulin up to five hours (Larsson et al. 1996, Segal et al. 1996, Pratley et al. 1996, Dagogo-Jack et al. 1996, Kolaczynski et al. 1996, Muscelli et a 1996, Ryan et al. 1996, Vidal et al. 1996).

One study did reveal an acute effect (within 30 to 60 min), as well as a dose dependant effect, and claims other studies fail to take into account the decline they observed in a control saline drip (Saad et al. 1998). A 72 hour fast with glucose levels clamped at basal levels produced no decreases in leptin levels (Boden et al. 1996). Four studies did show a delayed effect of insulin, resulting in an increase in leptin levels between 3 hours and 6 hours after insulin administration (Utriainen et al. 1996, Malmstrom et al. 1996, Fitsche et al. 1998, Schmitz et al. 1997, Tuominen et al. 1997). The study by Schmitz et al (1997) additionally shows that a hypoglycaemic clamp reduces leptin levels. Kolaczynski et al (1996) also showed the effects of a 72 hour glucose infusion (resulting in increase glucose and insulin) to increase leptin during the last 24 hours. Boden et al (1997) also showed an increase in leptin during a 72 hour euglycemic–hyperinsulinemic clamp occurred in a dose dependant manner. The same study showed that leptin did not change in response to hyperglycemia or high free fatty acids. Further support for more long term or chronic regulation of leptin levels comes from insulinoma patients who showed higher levels compared both to controls and themselves, following the removal of their insulin secreting tumors (D'Adamo et al. 1998, Popovic et al. 1998). One study shows that across various clamp protocols, varying between high and low insulin as well as euglycemic and

hypoglycemic, the relationship between infused dextrose and leptin remains constant (Wellhoener et al. 2000). Their regression analysis suggests that it is not serum insulin or glucose levels per se, but glucose metabolism that influences leptin levels. An additional study has shown that a state of induced insulin resistance and reduced glucose demand resulted in a blunted leptin response compared to controls in a hyperinsulinaemic –hypoglycaemic clamp, although the exact mechanism is unclear (Fruehwald-Schultes et al. 2002).

Leptin – Meaning of Deviation from the Norm

There is some question concerning the significance and long term effects of having particularly high or low leptin levels. Thus two possible interpretations can be made for levels that deviate from the norm: 1) that they represent a difference in sensitivity or responsiveness to leptin or 2) a dysfunction in production and/or clearance (James 2002). We have already discussed some deviations in leptin levels, based upon a functional change, such as seen in changes that occur resulting from negative or positive energy balance (see *Energy Balance* section). This section will deal primarily with what could be called basal leptin (at neutral energy balance). Basal leptin level show considerable variation, even among individuals with similar levels of adiposity (Sinha and Caro 1998). Low leptin levels may represent a high sensitivity, thus less leptin is required for leptin's effects, with high leptin levels representing decreased sensitivity with a higher levels of basal leptin needed for the effects to occur (i.e. leptin resistance). In humans about 10% of obese subjects have relatively low leptin levels, perhaps indicating a reduced rate of leptin production and it has been hypothesized that those

individuals may be more sensitive to exogenous leptin treatment (Ioffe et al. 1998, Friedman and Halaas 1998).

In Pima Indians relatively low leptin levels are predictive of later weight gain, suggesting that those individuals may underproduce the necessary levels to maintain a stable weight (Ravussin et al. 1997). This underproduction may essentially be sending an inappropriate signal of inadequacy, thus predisposing weight gain. A study by Lindroos et al (1998) showed a similar pattern with low leptin levels being associated with weight gain, however only in women with no parental history of obesity. Low baseline leptin has also been seen as predictive of weight regain in women following a 12 week dietary intervention at 5 month followup (Mavri et al. 2001). However, the weight maintainers showed a much higher decrease in leptin levels following the five week intervention, which the author's attribute to potentially regaining a leptin range with higher sensitivity, which the regainers failed to achieve. Additionally, post-obese individuals have been seen to have lower levels compared to match controls, after controlling for fat mass and fat free mass (Filozof et al. 2000). The authors suggest this may be related to the tendency for post-obese individuals to relapse after weight loss.

There is some evidence for variation in leptin sensitivities, with one dietary restriction study showing that the magnitude of initial leptin decreases correlates with the amount of weight loss when exogenous leptin administration is added to the dietary restriction (Fogteloo et al. 2003). This suggests that those individuals may be more sensitive to leptin's effects, thus better responders. Results by Tong et al. show that higher leptin predicts greater intra-abdominal fat accumulation at 5 and 10 year follow-ups (2005). Other studies have also shown that higher leptin was associated with an increase weight gain in adults (Kettaneh 2007, Van Rossum et al.

2003, Chessler et al. 1998). High leptin has also been seen to be associated with decreased physical activity energy expenditure over 5 years and increased risk of insulin resistance (Franks et al. 2007). In one study low leptin levels predict more weight loss in men during a weight loss intervention and during a maintenance period (Verdich et al. 2000). The authors suggest that perhaps those individuals with high leptin levels represent those with greater levels of leptin resistance. Other studies have also shown that low baseline leptin levels are predictive of weight loss (Shih et al. 2006, Torgerson et al. 1999). All of these studies support the idea of variation in leptin sensitivities, with high leptin (i.e. leptin resistance) predisposing to weight gain and low leptin predisposing to weight loss.

Some studies do not support the idea that leptin predicts changes in weight or fatness (Langenberg et al. 2005, Haffner et al. 1998, Hodge et al. 1998, Niskanen et al. 1997), regain after weight loss (Nagy et al. 1998), or both (Wing et al. 1996). The discrepancies between these studies could reflect from a number of factors, including the unique predispositions of some groups (e.g. genetic differences), study design, subject/population specific characteristics, and/or length of study (Jenkins and Campbell 2003, Filozof et al. 2000). Specific methodological criticisms have also been raised with various studies (e.g. Van Rossum et al. 2003) for their use of BMI or other height and weight, which may be poor proxies for fatness for such studies (Jenkins and Campbell 2003). Regardless of these problems, some of the evidence is suggestive that deviation in leptin levels may have physiological significance for energy regulation.

Leptin – Other Influencing factors

This section reviews some of the major variables that have been associated with variation and the regulation of leptin levels , beyond those which have already been discussed. Some of these variables (cold exposure, SNS, catecholamines, glucocorticoids, cytokines) have clear relationships with leptin, while others potential influences (high altitude, hypoxia, alcohol, smoking) are less clear. Only additional, well controlled studies, will be able to effectively tease these relationships.

Cold exposure leads to decreased leptin levels within 30 minutes of exposure, possibly as a result of sympathetic nervous system activation or decreased efficiency of the secretory pathways (Ricci et al. 2000, Penino et al. 2000). There is good evidence that the sympathetic nervous system does act to decrease leptin production, with experimental evidence showing that catecholamines inhibit leptin. Intravenous infusion with isoprenaline, the nonspecific β-adrenoceptor agonist, has been shown to decrease leptin levels within 3 hours (Pinkney et al. 1998, Donahoo et al. 1997). Intravenous infusions with epinephrine has also been shown to decrease leptin (Couillard et al. 2002, Carulli et al. 1999). *In vitro* studies with isoprenaline and epinephrine show similar results (Ricci et al. 2005, Scriba et al. 2000, Kosaki et al. 1996). These results show that catecholamines may acutely inhibit leptin, possibly being responsible for the cold induced decreases.

Perceived psychological stress (Otsuka et al. 2006) and posttraumatic stress (Liao et al. 2004) have both been associated with higher leptin levels. These responses may be mediated by glucocorticoids. *In vitro* studies have shown that cortisol increases leptin secretion in adipose tissue (Wabitsch et al. 1996). Injections of dexamethasone has shown a positive relationship with

leptin, consistently leading to increased levels (Lee et al. 2007, Eliman et al. 1998, Dagogo-Jack et al. 1997, Kolacznski et al. 1997, Masuzaki et al. 1997b, Papaspyrou-Rao et al. 1997, Larsson and Ahren 1996, Miell et al. 1996).

Various cytokines have shown a positive relationship with leptin, including interleukin-1α (Janik et al. 1997) and tumor necrosis factor-α (Mantzoros et al. 1997, Zumbach et al. 1997). This relationship may explain the high levels of leptin seen in sepsis (Bornstein et al. 1998). These relationships probably tie in with leptin's role with the immune system and inflammatory responses (see *Leptin – Regulator of other functions* section*)*.

Hypoxia has been shown to have an effect on leptin levels in some studies, although in various directions, with the nature of the relationship being unclear, possibly due to the multiple high altitude stressors (particularly cold exposure), the confounding effects of negative energy balance, or the use of various study populations (native, acclimated, or experimental) (see for review Sierra-Johnson et al. 2008, as well as the associated commentary). More research needs to be conducted in order to tease out these confounding effects.

Alcohol intake has been shown to have no association (Dammann et al. 2005, Togo 2000), a negative (Raben et al. 2003, Perkins and Fonte 2002 [women only], Rojdmark et al. 2001, Donahue et al. 1999) or a positive relationship with leptin (Roth et al. 2003, Mantzoros et al. 1998). All studies used BMI as their measure of fatness and the mixed results are probably a result of various study methodologies, subject characteristics, and the effects of confounding variables.

Studies on smoking have shown mixed results with some indicating that they are lower, even after controlling for body fat, (Reseland et al. 2005, Donahue et al. 1999, Mantzoros et al.

1998, Hodge et al. 1997, Wei et al. 1997) while others have found no relationship (Calissendorf et al. 2004, Klein et al. 2004, Perkins and Fonte 2002, Togo 2000, Yoshinari et al. 1998). Again these mixed results most are probably due to various study methodologies employed, over reliance on BMI, unique subject characteristics, and the effects of confounding variables tied in with smoking behavior.

In summary the variables that show a clear negative relationship with leptin are cold exposure, SNS, and catecholamines. Those showing a clear positive relationship include stress, glucocorticoids, and cytokines. Hypoxia, alcohol, and smoking all require further study to evaluate their effects, if any, upon leptin.

Summary and Conclusions:

This chapter has provided an overview of leptin and its functioning in human adults. It has specifically addressed its role in energetic regulation, with its primary function to act as a signal of energy deficiency, promoting multiple adaptive responses under such circumstances. Additionally, this chapter has addressed the major causes of leptin variation. The next chapter will address leptin variation further by examining the cross cultural variation in leptin levels.

Chapter 3: Leptin Comparative Analysis

This chapter presents comparative data on leptin levels from a variety of populations. I begin this chapter by discussing methodological issues related to population comparisons. I then present data from 25 male populations and 37 female populations. Data from these populations is analyzed to compare sex differences, various adiposity measures on leptin, and potential lifestyle effects.

Methodological Issues

By far the majority of studies on leptin have been conducted in westernized countries that could be considered in a chronic state of energy surplus. This section will examine population variation in human leptin levels, capturing a broader range of human variation. This endeavor involves a few problems including that the majority of studies rely upon BMI as a marker of adiposity or that they use a variety of ways to measure adiposity. As discussed in the *Male/Female Differences* section in Chapter 2 BMI is problematic when used as a measure of adiposity. Additionally it is poorly suited for use in cross population comparisons, as the relationship between BMI and true adiposity will vary across populations, due to differences in body proportions (Wells 2010, Leonard and Katzmarzyk 2010) or body composition (Fernandez et al. 2003). In this study fatmass is used for comparison because it tends to explain the greatest variation in leptin and using percentage body fat tends to under represent changes in adiposity in obese individuals (Bennett et al. 1997, Rosenbaum et al. 1996). The majority of these studies measure leptin in the morning, typically shortly after waking in a fasted state. Although this does

make such studies comparable, it may lead to inaccurate comparisons between groups if important variation exists in other segments of the 24 hour leptin profile or if other segments have greater physiological significance (Hickey and Calsbeek 2001).

Serum and plasma samples have been shown to have very high agreement, with a spearman correlation of 0.98 (Marshall et al. 2000, see Ma et al. 1996 for similar results). A study by Groschl et al. showed high agreement between K_2-ETDA, Li-heparinate, and fluoride treated samples (2000). However, compared to those, serum samples showed a higher coefficient of variation and poor recover of small (0.3 ng/ml) spikes in sample, while citrate treated samples all had lower values and also poor recovery of small and large (20 ng/ml) spikes in sample. Despite these reported problems with serum, the previous two studies do suggest that comparisons are valid; however comparisons with citrate treated samples, given their uniformly low levels, should be avoided. Although not exhaustive, measurements across three different assay kits, two RIA and one ELISA, showed correlation coefficients between kits of 0.95 to 0.98 (Frederich et al. 2002). These close relationships suggest that comparisons across studies are valid and that errors due to sample type (with the exception of citrate treated samples) or laboratory analysis differences are minimal.

Population Data:

Population leptin values are presented in Tables 3.2a and b. These studies were chosen because they represent healthy individuals drawn as representing their respective populations. Only studies where both percentage body fat and fat mass were given or could be calculated from the available data were used. A number of studies were drawn from the US as more studies

are done in the US compared to other areas, in order to fully capture US variation in values, and to provide a comparison with values typically represented in the literature (i.e. US populations) to those less seen and more poorly represented. The last point is particularly important given that US values may be seen as being a novel extreme in the evolutionary history of humans.

Results:

As can be seen from table 3.2a and b, average male values range from 1.13 ng/ml (Ache, SD = 0.37) to 17.4 ng/ml (Mexican American, SD = 18.5) and average female values range from 1.31 ng/ml (Evenki, SD = 0.28) to 60.8 ng/ml (Mexican Americans, SD = 46.6). In this across population data there is a significant difference in leptin levels between males and females (T-test, $t = -5.431$, $p < .001$).

Regression models were constructed to examine the strength of fat mass and fat percentage as predictors of leptin (logged, shown in Table 3.3). Models 1 and 2 were conducted on the entire data set, with sex included in the model. Model 1 had both fat mass and sex as significant predictors, while model 2 had fat percentage, but not sex as significant predictors. Model 2 masks the male and female differences due to poor overlap in values as can be seen in Figure 3.4a. In this figure all of the male values can be seen to group in the lower left hand corner of the plot (showing low logleptin and low percent body fat), while the female values typically show higher logleptin and higher percent body fat.

Models 3 and 4 were conducted on males only, showing that for males fat mass has both a higher standardized beta coefficient and a greater R^2 (model 3) compared to fat percentage

(model 4). Similar results were seen in females, with fat mass also being the better predictor (models 5 and 6).

Figure 3.2 clearly shows the sex difference and also suggests that the relationship between leptin and adiposity (represented by fat mass) may also be different between males and females, with female leptin levels being similar to males at lower levels and becoming increasingly different with increasing fat mass. The female populations show much more variation around the regression line, compared to the males (see figures 3.3a+b, females show 54% of the points outside of 95% confidence intervals compared to 28% in the males). This may indicate that female leptin is much more variable or that in females other variables besides fat mass are having an important impact on population variation. It may also be a product of the data resulting from more female data points or more of their points in the upper range of body fat mass.

Figures 3.3a and b also show that there exists considerable variation in leptin levels across populations. One of the possible explanations for this difference is that it is simply a reflection of differences in adiposity levels. As the r^2 shows (Male $R^2 = 0.778$, Female $R^2 = 0.629$) much of the variation can indeed be explained by differences in fatness levels, yet causes of variation beyond this remains to be explained. Leptin differences have been linked with lifestyle in other species, with captive baboons having levels three times higher than wild baboons (Banks et al. 2001). Although some populations below the 95% confidence interval could be considered subsistence level populations (i.e. Ache or the Buryat) others such as the elderly german population or Rosenbaum's 2001 US population (in males) have considerable qualitative difference in background and lifestyle, making any conclusions about causes of

variation difficult. Additional analysis tested for lifestyle related differences by coding the populations into two groups, one that lives in urban areas or are highly involved in the market system and a second one who live in rural centers or are considered subsistence level populations (see tables 3.2a and b for coding of each population). These designations were based upon the information provided in the source article and are considered fairly broad classifications. Regression models were created using leptin as the dependant variable and fat mass and lifestyle as independent variables. The results showed lifestyle to be non-significant in males ($p = 0.134$, model 7 in table 3.3) and significant in females ($p = 0.038$, model 8 in table 3.3.). However such effects are highly tenuous as the significance in females disappears in if we remove the low evenki data point and the males become significant if we remove a high south African rural population point that is relatively ill defined in its source article concerning it's subsistence base (for the data point distribution see figure 3.5 for leptin plotted against fat mass with the data stratified by lifestyle). As such the larger lifestyle effects on cross population leptin remains ambiguous. Additionally, whether or not these population differences in leptin levels are functionally meaningful has yet to be determined. More studies in non-westernized populations, particularly those from rural and subsistence level populations, must be conducted to fully understand the range of leptin variation and its functional significance.

Summary and Conclusions:

In conclusion, these findings show considerable cross population variability in leptin levels that is not entirely explained by differences in adiposity. Cross population sex differences exist, with females have higher levels, a pattern consistent with previous within population

findings. Lifestyle may play a role in the observed cross population differences, but more studies from rural, subsistence level populations are required. Chapters 5 and 6 will address leptin variation further, taking a more in-depth look within specific populations, the Tsimane' and the Philippines.

Chapter 4: Background on the Tsimane'

The Tsimane' are a population of indigenous Amerindians from the Ballivián province of the department of Beni in lowland Bolivia. They have approximately 50 villages along the Maniqui River, with 30 villages near other rivers (Gurven 2000). They are considered to be forager-horticulturalists due to their practice of swidden agriculture (i.e. slash and burn) and high dependence upon the forest (Byron 2003). Since the 1950's they have become more settled due to missionary influence, the establishment of schools, and increased market involvement (Chicchon 1992). This chapter provides a brief overview of Tsimane' history, cultural, ecology, subsistence, demography, and market involvement. (For a more thorough overview see Byron 2003, Reyes-Garcia 2001, Chicchon 1992).

Historical Overview

Archaeology and Early History:

In the pre-columbian era, the land that now comprises the department of Beni was dominated by tropical forest chiefdoms (Reyes-Garcia 2001, Jones 1980). Archaeological features from surrounding areas (and some within the Tsimane' territory) include extensive furrow-ridge fields, raised fields, mound fields, causeways and canals (Heckenberger and Neves 2009, Erickson 2008, Plafker 1963, Denevan1963a). Metraux suggests that these features were made by a population that preceded the Tsimane' or may even have been an ancestral group to them (Metraux 1942). The size and complexity of these systems suggests a more intense level of agriculture than the Tsimane' or other indigenous groups currently living in the area have

historically been known to utilize (Plafker 1963). Unfortunately, in-depth archaeological excavations have not been conducted in Tsimane' territory (Byron 2003). These archaeological features underscore the idea that the Tsimane' do not present a picturesque image of a people living in a pristine untouched environment, but rather they are a people with a complex past occupying an ever-changing environment (Clarkson 2001).

Although the early Spaniards carried out expeditions into the area, they failed to set up colonies (Reyes-Garcia 2001). The first historical reference to the Tsimane' was made by the Franciscan priest Gregorio de Bolivar in 1621 (Chicchon 1992, Metraux 1942). Another Franciscan priest, Francisco del Rosario, also mentions the Tsimane' after visiting the area in 1666, estimating their population to be 2,000 to 3,000 (Chicchon 1992, Metraux 1963). During this time various religious missionaries attempted to work in the area, with the Jesuits being the most successful. One of the lasting effects of the Jesuits' tenure is the introduction of rice, one of the area's main staples (Piland 1991). In 1767 the Jesuits were expelled and two short-lived Franciscan missions were established in the mid 1800's (Ellis 1996). Despite missionaries being in the area for over 100 years, the Tsimane' were among the groups in which their efforts to Christianize failed. Chicchon theorizes that this was due to the Tsimane's lack of central authority and semi-nomadic lifestyle in which they occupied a wide area (Ellis 1996, Chicchon 1992). After missionaries left the area, evidence suggest little Tsimane' involvement in Bolivian commerce (such as the rubber or quinine trade) or politics until the middle of the 20th Century. However, it should be noted that following the late 1800's migration into the Beni region increased as the international markets for Bolivian resources increased;, it is only the extent of Tsimane' interaction with these changes and its effects upon them that is unknown.

It is unclear when the Tsimane' became a distinct social group (Byron 2003). The Tsimane' are linguistically related and culturally similar to a neighboring group, the Mosetene (Riester 1994, Chicchon 1992, Metraux 1963, Denevan 1963b). Ellis notes cultural similarities between the two groups and the fact that the Mosetene are the only other group that the Tsimane' identify as "real people" (Byron 2003, Ellis 1996: 17). Their linguistic relationship coincides with work on mitochondrial DNA, suggesting a higher degree of relatedness between them than compared to other groups (Bert et al 2001). Their separation has been hypothesized to be the result of the Jesuits successfully reducing the Mosetene into missions during the seventeenth century, while the Tsimane' successfully resisted such efforts (Ellis 1996, Chicchon 1992). Their close genetic and cultural relatedness may also be due to inter-marriages that have occurred in the past and continue today (Ellis 1996).

Regional Economic Industries: Cattle Ranching and Logging:

Cattle ranching became an increasingly important economic enterprise in the 1900's once the rubber market collapsed (Byron 2003). Following World War II, the cattle industry expanded rapidly due to the use of military surplus cargo planes for transport, a rise in national beef prices, and agrarian land reform that increased private ownership of large ranches (Byron 2003, Piland 1991, Jones 1990). As a result of this expansion, the Tsimane' worked on and provided agricultural goods to ranches, and began to alter their settlement patterns to help support these endeavors (Piland 1991).

In the 1970s logging companies turned to the Beni following the depletion of mahogany from the department of Santa Cruz (Piland 2003). Conservation efforts prompted the creation of

the Chimane Forest Reserve in 1978 in order to protect the resource. Due to pressures from timber companies and a poor economy, part of this reserve was later opened to logging in 1987 (Jones 1990). Indigenous groups, including the Tsimane' were not a part of this decision and had increasing conflicts (including violence) with loggers as well as experienced the adverse environmental effects they were causing (Reyes-Garcia 2001, Jones 1990). Despite these problems, the loggers provided jobs and for a period after its creation, the Tsimane' council received monetary benefits from them (Reyes-Garcia 2001).

Both cattle ranching and logging remain important economic forces in the area today. They continue to act as sources of jobs for the Tsimane' and influence them in both positive and negative ways.

Post-1950's Missionary Influence:

Along with the economic changes also came renewed missionary efforts aimed at the Tsimane', starting with Catholic missionaries at Cara Cara in 1953. This mission was later moved to Misión Fátima, where a Tsimane' community still resides today. The missionaries tried to introduce cattle ranching and the cultivation of cash crops (cacao and coffee), which failed to catch on beyond those Tsimane' living in the immediate area (Chicchon 1992). Community members today have a large communal field of rice, look over a small herd of cattle, and attend mass every Sunday (Winking 2005: 37).

Evangelical "New Tribes" missionaries (Protestants) have also had an important influence upon the Tsimane' since the 1950s. They are based at Horeb (3 km from the city of San Borja) and in the Tsimane' community of La Cruz. They have worked to improve education

and medical care. They conducted linguistic studies of the Tsimane' language, resulting in the creation of a Tsimane' dictionary and the translation of the Bible into Tsimane' (Reyes-Garcia 2001, Winking 2005). They provided the support and infrastructure that helped to enable Tsimane' political organization and aided in the formation of the Gran Consejo Tsimane' in 1989 (Chicchon 1992). They have helped to protect the interests of the Tsimane' in dealings with both traders and loggers (Chicchon 1992). Today they broadcast Christian radio programs, offer basic medical services (in a work for care program), and train Tsimane' bilingual school teachers (Winking 2005).

Politics:

Early accounts suggest that the Tsimane' had little to no formal political organization, lacking distinct chiefs (Byron 2003, Riester 1994). Traditional informal leadership typically consisted of elders or outspoken Tsimane', and more recently teachers (Byron 2003, Chicchon 1992). This has changed since 1987, as the Tsimane' started to join other indigenous groups in various political organizations (Reyes-Garcia 2001). In 1989 The Gran Consejo Tsimane', a formal political leadership structure, was created to serve as representation and to act as a voice for the Tsimane' due to pressure on natural resources, encroachment on territory, and quarrels with loggers and traders (Huanca 1999, Ellis 1996, Chicchon 1992). However, its representativeness, political activity, and ultimately effectiveness are questionable (Reyes-Garcia 2001). Additionally, its leadership has been static and the participation of non-council Tsimane' is limited.

Following the establishment of the Gran Consejo there has been some movement within communities towards electing community leaders called “corregidores”. However, formal within community politics still tend to either be nonexistent or exist with little to no real power (Reyes-Garcia 2001). Thus, Tsimane’ households and family clusters still remain very independent.

Given the size of the Tsimane’ population they could serve as an important voting bloc, particularly in local elections. While I was in Puerto Yucumo elections for local political offices did occur, with voting being held in that community and people from other villages converging there to participate in voting and to visit. Food was brought to the community to encourage participation and some promises of goods were made from politicians to communities, but the Tsimane’ have yet to truly organize their voting and make cohesive political use of it.

Contemporary Life:

Urban Centers:

The two closest urban centers are the towns of San Borja and Yucumo. San Borja is the more important of the two in the region, acting as the local center of political and economic activities. As of 2001 it had a population size of 16,723 (up from 3,200 in 1970) (Instituto Nacional de Estadisticas de Bolivia). It has an airport, nearby hospital, and is a primary stop for buses into and out of the region. It is also where the offices of the Gran Consejo Tsimane’ are located. Those living in communities closer to these urban centers show more frequent day trips for cash sales or to buy supplies, while those farther away typically rely more on barter with

traders (Byron 2003). The Tsimane' are often treated poorly in economic transactions and at the hospital, both currently and historically (Byron 2003: 115, Chicchon 1992).

Villages:

The Tsimane' live in villages of 8 to 100 households, with a census of 45 villages along the Maniqui putting the mean village size at 93 individuals (median = 75) (Winking 2005, Gurven 2000). Thirty villages have schools where the students learn to read and write in both Tsimane' and Spanish (Winking 2005). Villages show variation in river access, surrounding game densities, and access to market goods.

Traditionally the Tsimane' have been semi-nomadic living in small (< 50 individuals), isolated groups of extended family households (Reyes-Garcia 2001, Ellis 1996, Chicchon 1992). Metraux hypothesized that they live scattered throughout territory for fear that any large concentration of people at a given point would soon exhaust available natural resources (Metraux 1963: 491, Metraux 1942: 20). Since the 1980's they have become more settled, with the establishment of schools, latrines, water pumps/wells, community-owned (or -used) radios, solar panels, and boat motors all serving to establish greater cohesion and continuity in communities consisting of a larger number of unrelated households (Tanner 2005, Reyes-Garcia 2001, Chicchon 1992). Increased colonization has also caused the movement of Tsimane' communities closer to rivers and away from roads (Reyes-Garcia 2001). However, more isolated communities still tend to be smaller (Reyes-Garcia 2001).

Extended families can often be found living in clusters, formed with an older couple and their married children living in relatively close proximity (Piland 1991). Clusters offer some

pooling of resources, protection, and task specialization (Piland 1991). Task specialization can be seen in that the most successful hunters are often the least productive farmers and vice versa, often complimenting each other and sharing in consumption of goods (Piland 1991). Individual families typically live around an open courtyard, usually with an open walled thatch house. In some of the closer villages some houses are walled and have doors with locks. In more remote villages houses have 2.5 walls, with a quarter of the houses having no walls (Godoy 2007). The majority of households have no electricity. Water is often from the river, but may also come from wells, streams, or water pumps.

Activities and labor within a household are often divided based upon gender and age (Winking 2005, Rucas 2000, Piland 1991). Men are most often involved in hunting, fishing, wage labor (cattle ranching/logging), felling trees and clearing underbrush, and certain productive activities (i.e. making bows, arrows, canoes, and houses). Women are involved in food processing (including making chicha, maize or cassava beer), clothes washing, fetching water, gathering, horticulture (weeding, harvesting, and attending to agricultural overproduction when it occurs), childcare, and certain productive activities (weaving items such as marico bags, floor mats, fans, baskets). Both genders are involved in the production of *jatata* (palm roofing panels) and horticultural activities (burning, planting, weeding, and harvesting). Female children are often helping with household duties (e.g. fetching water, child care), while male children spend more time on fishing and hunting, usually for personal consumption (Aiello personal communication). Many boys and girls also attend schools up to a maximum of grade five in village, with most children averaging much less than that (Byron 2003). Older male children (~12 years of age) may also tend to their own small agricultural fields (Aiello personal

communication). Some of the gendered tasks result in different levels of household production. For example families with adolescent sons produce more plantains, while those with adolescent daughters produce more rice (Piland 1991: 107).

One of the most important Tsimane' customs is visiting other households, what the Tsimane' refer to as *sobaqui* (Huanca 1999). Ellis defines *sobaqui* as "to travel with a purpose, to visit someone, see something, hang out or wander for the sake of doing so" (Ellis 1996: 25). A range of activities can qualify as *sobaqui*, including daily visits with people close by, visiting while traveling to somewhere else, or longer, planned long distant visits to relatives. These visits serve as an important aspect of social life, allowing people to exchange goods, cement relationships, gossip, pass on news and other information. These visits are such an important part of Tsimane' life that some studies have experienced temporary attrition due to the practice (Reyes-Garcia 2001). During such visits chicha plays an important role. Ellis highlights the importance of chicha, suggesting that most people rarely consider visiting kin unless they are sure of a steady supply of beer (Ellis 1996: 153). Ellis consider it essential, allowing loss of *tsicadye'* (socially restricting embarrassment), giving people more freedom to talk, sing, dance, and make music (Ellis 1996: 153). Those awaiting visitors from father away spend four or five day's prior preparing chicha (Ellis 1996). Ellis argues that is importance is both physiological and psychological, allowing people to become close to kin, share knowledge, intimacies, and socializing (Ellis 1996: 154).

Ecology and Subsistence

Basic Ecology:

Typical of Amazonian ecologies, the department of Beni experiences wet and dry seasons that show variation in rainfall and to some extent temperature (See figures 4.1 and 4.2 for a year profile during the research period). Godoy et al. compiled data on rainfall from 1944 to 2005 and showed a mean annual rainfall of 1743 mm (SD = 494), with a minimum of 872 mm and a maximum of 3907 (Godoy 2008, see figure 2 on page 28 for averages by month). Piland compiled similar data on temperature from 1948 to 1978 and showed an average temperature of 25.9ºC, with November being the hottest month (27.6ºC) and July being the coolest (23.2ºC) (Piland 1991). Godoy has shown that climatic conditions have been relatively consistent over time, with no secular changes occurring in temperature (1973-2005) or rain (1943-2005) (Godoy 2008).

The wet season runs from November to April and can be characterized by high rainfall and humidity. The dry season runs from May to October and can be characterized by dry, cool weather. Rains peak during January and often flooding occurs. During the rainy season travel to San Borja becomes difficult, with bikes and motorcycles becoming less reliable forms of transportation, while walking often becoming the most reliable option for close villages (Reyes-Garcia 2001). River travel also becomes more difficult due to strong currents and increased river debris. Rainfall has also been noted to decrease foraging productivity, creating problems using hunting dogs and making fishing water turbid, thus decreasing the necessary visibility (Godoy 2008). During the dry season river travel may also be difficult at times due to low river levels, which increases the risk of running aground on sandbars (Byron 2003). Temperatures may

sometimes drop significantly due to the "*Sur*" or *Surazos*, cold wind that come from the southern Antarctic Region. This usually happens during the dry season, but may occur at anytime. During the Sur temperatures may drop down to as low as 6ºC and last from one to ten days (Piland 1991).

Hunting:

Hunting occurs all year long and may be planned or opportunistic (Chicchon 1992). It is primarily done by men, who hunt most commonly with bow and arrow (Winking 2005, Gurven 2000). Rifles and shotguns are preferred, but those living farther from urban centers have more limited access to ammunition (Winking 2005, Chicchon 1992). Due to their limited availability, firearms are sometimes shared in exchange for a share of hunted meat (Chicchon 1995). Machetes and dogs are also sometimes used and may even be utilized by women at times to hunt (Chicchon 1992, Aiello personal communication).

Typical hunting trips involve one to three men traveling a few kilometers in the area surrounding a community and usually last a couple of hours. However, larger trips may involve greater numbers of people and multi-day treks (Winking 2005). Abandoned house gardens attract animals and can often serve as hunting sites (Piland 1991). Following a hunt, animal meat is often preserved by smoking it or salting and sun drying to make *charqui*, a jerky (Byron 2003). Types of game hunted include peccary (*Tayassu tajacu* or *T. pecari*), tapir (*Tapirus terrestris*), deer (*Mazama americana*), rodents (*Agouti paca*), monkeys (*Alouatta caraya* or Saimiri sciureus), armadillo (*Dasypus novemcinctus*), coati (*Nasua nasua*), and various birds

(Winking 2005, Rucas 2004, Chicchon 1992). Animals start to gain weight in May, spurring hunting during that time (from May to July) (Godoy 2008, Godoy 2004).

Some village-related variations in hunting patterns have been observed. Those people living in communities closer to San Borja tend to hunt close to the settlement where faunal resources are depleted (Chicchon 1995, Chicchon 1992). Over time this practice has shifted the types of animals successfully hunted in this area from large animals to medium sized rodents or edentates. More distant and inland villages typically have greater hunting returns, due to less overhunting by overpopulated villages (Rucas 2004). However, those living in farther away villages also spend more time hunting and typically travel farther distances during hunts (Chicchon 1992).

Chicchon uses Puerto Mendenz as an example of a village that has experienced a change in hunting as a result of land use restrictions brought on by its proximity to San Borja and settlers. Due to its location the majority of land consists of secondary forest, agricultural fields, and fallows. Habitat destruction and overhunting have caused a low availability of larger animals. The residents of Puerto Mendez respond to this challenge by hunting small animals common to secondary growth areas (such as rodents) and by intensifying other forms of resource acquisition, such as fishing, intensifying agricultural production, and increased market involvement with the produced surplus (Chicchon 1992)

The Tsimane' recognize a decrease in the density of available animals due to the use of guns and from the movement of non-Tsimane' into the area (Godoy 2006). Despite this recognition an analysis by Godoy et al. shows no change in Tsimane' wildlife consumption patterns in their 2002-2006 annual surveys (Godoy et al. 2009).

Fishing:

Fishing occurs all year and is the most important to source of protein in the Tsimane' diet, providing over 50 % of meat in the diet (Byron 2003, Metraux 1942). Based upon dietary recalls, Byron reported that the Tsimane' utilize 37 different species of fish, with *sábalo* (Prochilodus nigricans) being the most common (Winking 2005, Byron 2003). Men fish with bows and arrows, while men, women, and children engage in hook and line fishing, machetes and in some communities fishing nets (Chicchon 1992, Gurven 2000). During the rainy season fishing with hooks is the only practical way to fish (Chicchon 1992). During the dry season, the river is low and fish are migrating upriver, allowing for higher yields and increasing the importance of fishing even more (Chicchon 1992). At this time people may move closer to the river for fishing (Chicchon 1992). The low, calmer (less turbid) river allows for Barbasco fishing, where a portion of the river is blocked off and poisonous vines are used to slow the fish. Barbasco-type fishing is more common in the isolated communities living farther away from San Borja (Byron 2003). Much like hunted meat, fish is preserved by salting and sun-drying or smoking.

Settlements vary in the proportion of fish that make up the meat in their diet (Byron 2003) and in the absolute level of yields (Chicchon 1992). The reasons for this variation are complex and seem to involve individual community variables, such as access to alternative food resources, opportunity for Barbasco fishing, and the ability to fish effectively in the wet season. Understanding this variation is further complicated by the likely underreporting of fish caught by children (Chicchon 1992).

Gathering:

Gathering can occur in an opportunistic manner or as a planned event and is performed by men and women of all ages (Chicchon 1992). Commonly gathered items are fruits and berries, honey, and eggs (birds and reptile). Winking reports that foraged foods rarely contribute significant proportions to daily calories, however, amounts eaten are often hard to quantify, as items are consumed as gathered (Winking 2005, Byron 2003). Fruits start to ripen in May, but gathering becomes especially important between October and December when most tree species are fruiting (Godoy 2008). Some evidence suggests some unsustainable resource use (turtle eggs and honey) (Chicchon 1992). Beyond food, gathering is also done for firewood, medicine and for supplies to make such items as woven mats, fans, bows, and arrows.

Agriculture:

Tsimane' agriculture based on slash and burn methodology provides an important contribution to the Tsimane' diet. Two thirds of a typical field contains rice and plantains, providing the bulk of the diet and some surplus (Chicchon 1992, Piland 1991). Rice is the main cash crop, in which surplus is specifically grown to be sold (Reyes-Garcia 2008). Because of this it is planted in higher quantities in communities better able to trade it (Vadez et al. 2004). Plantains may also be a prominent commodity in some communities (Byron 2003). Sweet manioc and maize are also very common and are usually consumed in the form of chicha (Winking 2005). Maize is also fed to poultry or sometimes sold (Piland 1991). Other crops may include sugar cane, sweet potato, watermelon, peanuts, pineapple, and papaya (Piland 1991). Households have one to six active agricultural fields, with an average of three (Piland 1991).

Measured average field size is 2815 square meters, with reported field sizes likely being overestimated (Piland 1991).

Agricultural plots are cleared May through August, burned from September to October, and then planted (Reyes-Garcia 2001). The Tsimane' plan burning as close to the wet season as possible in order to maximize crop yields (Godoy 2008, Piland 1991). Crops are harvested in the wet season, usually February and March (Gurven 2000). Each field is typically cultivated for two to three years and then set aside for forest regeneration. Plantains are planted and harvested all year round as needed (Gurven 2000), as plantains as well as manioc do well with little rain, while growing rice is more rain-sensitive (Godoy 2008). The same plots are rarely used for more than two seasons before allowing to fallow and then they may be used five years later (Reyes-Garcia 2008).

Typical Amazonian soils are characterized by being acidic, with low fertility and suffering from varying degrees of aluminum toxicity (Piland 1991). The only soils suitable for continuous crops are those on alluvial river terraces, such as those along the Maniqui river. However, it should be noted that much ecological variation in land exists in Tsimane' territory, as recognized by both researchers and the Tsimane' themselves (Piland 1991). This variation may affect productivity with some community differences in the proportions of different items grown due to differences in soil compositions. In a community with more clay dominated soils, a lower percentage of the fields were planted with manioc compared to communities with sandier soil, where manioc will thrive better (Piland 1991: 113). Also, some older, closer communities may lack sufficient agricultural fallows due to encroachment from loggers, ranchers and colonist farmers (Godoy 2006, Vadez 2004, Piland 1991). This limitation may cause a shortening of the

length of the fallow period in available plots, a signal of agricultural intensification (Godoy 2006, Vadez et al. 2004).

In addition to their horticultural practices, the Tsimane' also raise some domesticated animals. These include chickens, pigs, ducks, goats, and cattle. These are considered a source of wealth, rather than of meat, and thus are often sold, traded, or used during special occasions (Byron 2003). Chickens are the principle livestock (Byron 2003: 223). Livestock provides only a relatively small percentage of the meat in the diet, 6% according to Byron (2003: 224). Non-domesticated wild animals such as ducks, monkeys, rhea, pacas, and tapir are also kept as pets and are eventually eaten (Piland 1991).

Marriage and Demography:

Tsimane' Marriage:

The Tsimane' have a preferential system of cross-cousin marriage in which the man marries his mother's brother's daughter (Godoy 2005). This has been reported to occur in 75% of cases overall and up to 90% in more remote villages (Patel et al 2007). This tradition of cross-cousin marriage acts to "create a thick and wide web of relatives linked by descent and by marriage" (Godoy 2005: 143). Men start looking for spouses in the late teens (courting an average of just above two), with any woman past menarche being considered eligible (Winking 2005, Chicchon 1992). At that time it is considered that men are competent at the activities that will enable them to sustain a family, such as working in the field, hunting, and fishing (Chicchon 1992). The Tsimane' lack formal marriage ceremonies and couples are considered married when

they sleep together in the same house. Divorce occurs most commonly within the first year, ending 20% of marriages (Winking 2005).

Post-marriage residence tends to be matrilocal, with the couple building a house near the wife's family (Winking 2005). This is followed by neolocal residence after the birth of the first child (Godoy 2008). Winking reports the median age at marriage is 21 years for males and 16.5 years for females, while Godoy states 16 years is the typical age of unions in males and females, with females forming unions as young as 13 (Godoy 2008, Winking 2005).

Very few Tsimane' marry neighboring Amerindians and almost no-one marries non-Amerindians, with a sample from Godoy showing only 1.40% (5 people) belonging to another ethnic group (Godoy 2008, Godoy 2006). This may be related to Tsimane' taboos about intercourse with non-indigenous which is believed to lead to physiological harm, including developing Leishmaniasis (Ellis 1996). Although practiced traditionally, there has been a movement away from polygynous marriages (Reyes-Garcia 2001). Only one male individual in my data set was involved in polygyny and it has been estimated to occur at a rate of 6% or less (Godoy 2008, Winking 2005, Byron 2003).

Men (n=34) report an average of 1.4 sexual partners prior to first marriage (Winking 2005). Some culturally defined sexual prohibitions also exist, prohibiting sex with wives during pregnancy until six months postpartum and before labor-intensive activities (Huanca 1999). The extent to which these taboos are actually followed is currently unknown. Another understudied issue is the degree to which extramarital relations occur in the Tsimane'. This is difficult to study accurately due to various methodological and subject issues (Blow and Hartnett 2005). Winking reports that the Tsimane' view such affairs by men as directly harmful to their children,

leading to sickness and death (Winking 2007). He found that 31% of men (n=29) had at least one affair in the first five years of marriage and such affairs tended to be concentrated in that first five year period (Winking 2007). When asked, 89% of women thought that some Tsimane' men were having sex with Tsimane' women who were not their spouses while 81% reported that they thought some Tsimane' men were having sexual relations with non-Tsimane' or with prostitutes (n=36). When asked, 62% of men thought some Tsimane' men were having sex with non-spouse Tsimane' or non-Tsimane and 58% reported they thought some Tsimane' men were having sexual relations with prostitutes. Knowledge of sexually transmitted diseases is limited with only 47% of women (n=37) and 17% of men (n=24) reporting a belief that a disease can be transmitted sexually. This situation is particularly alarming given the reported beliefs about the occurrences of male extra-marital affairs, particularly with prostitutes. A more thorough investigation of Tsimane' sexual health would elucidate these matters.

The Tsimane' largely choose their own mates, with some parental influence (especially for girls) (Godoy 2008). Evidence suggests that the Tsimane' practice positive assortative mating, choosing mates that resemble them in select characteristics, which are age, knowledge, wealth, school, height and smiling (Godoy 2008). Gurven et al. also reports that time spent in productive activities is also subject to positive assertive mating (Gurven 2009). It is unknown what other characteristic may or may not be subject to positive or negative assortative mating in the Tsimane'. When looking for a mate, men put value on "women who know how to weave, wash clothes, cook wild game, prepared fermented beverages and spin cotton" (Godoy 2008: 205). Women look for men who "farm and hunt well, who know how to fish with a bow and arrow, who enjoy drinking fermented beverages, and who work hard" (Godoy 2008: 205). More

recently women have also started to look for men who have some fluency in Spanish, as it increases their economic opportunities (Godoy 2008). These preferences mirror similar findings of Gurven et al., who found that aspects of food and economic productivity were highly valued in mate preferences (Gurven 2009).

Demography and Reproduction:

The age structure of the population is a relatively young one, with over half the population (51-57%) being below the age of 15 years (Reyes-Garcia 2008, Supa et al 2007: see page 47, figure 2 for a population pyramid). This pattern is reflected in the relatively few people at the higher end of the age structure, with 10-11% percent being above 45 and only 3% being above 70 (Reyes-Garcia 2008, Supa et al 2007). The Tsimane' have an annual population growth rate of 4.86% from 1971 until 2002 (Reyes-Garcia 2001). Godoy suggests that this may be from a decline in mortality rates due to better health, with one of the causes being vaccinations (Godoy 2006). Little change was seen in mortality patterns from 1950 to 1989, however following 1990 life expectancy at birth improved from 45 to 53 years (Supa et al 2007). This change differed from classical epidemiological transition models in that the largest reduction was seen in deaths during middle to late adulthood, rather than during infancy or childhood (Supa et al 2007). The main causes of mortality are infectious disease (greater than half the deaths), accidents, and violence (Supa 2007). Mortality rates during infancy to middle adulthood are two to four times higher among remote villages compared to more acculturated ones (Supa et al 2007). Supa hypothesizes that this may be due to infants and children in remote

villages not being able to receive medical help as quickly as those in closer villages (Supa et al. 2007).

For the Tsimane', marriage is tied to reproduction and having offspring; Godoy writes that the "Tsimane' cannot conceive of a union without children" (Godoy 2008: 205). It is not a surprise then that women are considered marriageable following menarche. Age at menarche for the Tsimane' is fairly young, reported to be 12 to 13 by Byron (Byron 2003) and 13.9 by Walker (Walker 2006). The first birth typically occurs around the 2nd year of marriage, with an average inter-birth interval of 2.5 years (Gurven, Kaplan, and Winking 2004). The age at first birth for females is 18.6, while for males it is 23.0 (Walker 2006). The overall lifetime fertility is 5.1 children per woman (Byron 2003), while the total fertility rate is 6.5 children per woman (Gurven, Kaplan, and Winking 2004).

The average annual birth rate of two communities is 6.1% (Byron 2003). There is little contraceptive use and in fact people generally feel that they have little control over fertility (Godoy 2006). My sample reported 79% of women have never used contraceptives, 17% are currently using plants, 2% are using injections, and 2% have used a form of contraceptives in the past (n=47). Byron reported that during any quarter of collection 15-19% of her sample of women were pregnant (Byron 2003). For these women there is relatively little formal prenatal care, with a few women receiving iron tablets (Byron 2003). Typically a female relative attends the birth, usually a mother or mother-in-law or someone particularly skilled and experienced at birthing (Byron 2003). Byron reported that in one community (but not in another one) some husbands attended (Byron 2003). There are some food taboos surrounding pregnancy, typically

due to beliefs that certain foods will affect the health of the child or the difficulty of labor (Huanca 1999, Chicchon 1992).

Ninety percent of pregnancies result in live births (Byron 2003), although this number is probably an overestimate given the difficulties of accounting for early fetal loss (Vitzthum 2001). In Byron's study 2% of the total pregnancies ended in induced abortions and 6% ended in spontaneous abortions (Byron 2003). Supa et al has comparable numbers for 1990-99, estimating 4.8 % of pregnancies ended in miscarriages (Supa et al 2007). The Tsimane' have an infant mortality rate of 134-137/1,000 live births (Supa et al 2007, Byron 2003). Most likely due to the chance of early mortality, the Tsimane' typically do not name their children until after the first year (Supa et al 2007, Byron 2003, Scheper-Hughes 1987).

During Byron's study 17-23% of the women were lactating (Byron 2003). Tsimane' women believe that having an adequate supply of breastmilk is important for the health of their child and complain about not being able to feed their babies sufficiently (Gurven 2009). Breastfeeding occurs up to three years or when their next pregnancy occurs (Byron 2003). The pattern of on-demand breastfeeding among the Tsimane' is most likely one of the main determinants of their inter-birth interval (Wood 1994).

Market Involvement:

Market Activities:

One of the characteristics that draws researchers to the Tsimane' is their relationship to the market system and the large amount of variation, both between and within communities, in their levels of exposure and involvement (Byron 2003, Godoy 2001, Reyes-Garcia 2001).

Previous research has shown that communities closer to San Borja show higher monthly incomes, greater income fluctuations across collection periods, higher female incomes, and higher variability in female market involvement (Byron 2003). Seasonal and monthly fluctuations in market involvement also exist, related to mobility (rainy/dry), agricultural harvest periods, and wage labor opportunity.

The majority of the Tsimane' are not fully involved in the market system, with irregular incomes that would not adequately function as a sole means of support. Most of the Tsimane' retain a subsistence base firmly rooted in horticulture and the forest, largely living in a state of autarky (Byron 2003, Godoy 2001). Godoy reported that 74.88% of adults reported no earnings from wage labor and 56.40% reported no earnings from the sale of goods when asked about the previous fourteen days (Godoy 2009). Despite this limited dependence and involvement, the Tsimane' relationship with the market is not necessarily new. Before regional expansion into national and international markets, via addition of roads and air transportation, a large portion of the areas agricultural goods and building materials would have been produced by the Tsimane' (Chicchon 1992:204, Piland 1991:97). These items would have typically been exchanged through barter rather than cash. This practice indicates that the Tsimane' involvement in the market is actually long-standing, with the trade of forest goods (Byron 2003, Rioja 1992). However, today there is not so large a market for non-timber forest goods (besides jatata) and the Tsimane' involvement with the market has diversified to include other activities (Byron 2003).

The Tsimane' have a number of options for earning money and participating in the market system. These are through various types of wage labor and through the sale (or barter) of goods. Wage labor opportunities for males are available in logging, cattle ranching, jatata

extraction for traders, or agriculture (Byron 2003: 95). Most of these jobs are only seasonally available, with higher opportunities for income in the dry season. Some of these jobs are also only regionally available, with jatata extraction only being important farther upriver (Godoy 2004). Women do not typically engage in wage labor and subsequently have relatively low incomes (Byron 2003). A small number of Tsimane' are also involved in salaried work, such as being school teachers (Godoy 2007).

According to Byron the sale or exchange of goods is a much more important source of market income compared to wage labor (Byron 2003). Items typically sold include rice, plantains, maize, manioc, fruits, domesticated animals, eggs, honey, firewood, and jatata (Godoy 2007). Much like wage labor, cash income from sales shows high seasonality. Sales for Tsimane' good, such as woven mats, increase in the weeks before the festival of San Borja (October 10-12) (Godoy 2004). Sale of agricultural goods, such as rice, follows the harvest season. People living in communities close to San Borja are able to bring their goods directly to market. However, those living farther away often deal with itinerant traders instead, often receiving far less than fair market value for their goods.

One of the unique and primary goods in which the Tsimane' manufacture and trade is Jatata thatch roof panels. Jatata is considered a high quality roofing material; it offers good ventilation, is suitable for various types of roofs, and repels insects (Rioja 1992). Jatata roof panels are produced by both men and women, mainly in communities located upriver, because the raw material (palm leaves) necessary for weaving the thatch panels are located in heavily forested areas and requires two days to collect (Rioja 1992). Traders usually come with boats

full of good for barter that they exchange for jatata. They tend to be highly exploitative, selling exchanged jatata in San Borja for four times the bought value (Ellis 1996, Rioja 1992).

Consumption:

Most Tsimane' immediately dispose of cash, with very little savings (Byron 2003). Many of these immediate purchases consist of consumables, with market foods being the most frequently purchased good by the Tsimane' and is 29% of the items in their diet (Godoy 2005, Byron 2003). Typically purchased market foods include sugar, salt, animal lard, vegetable oil, white flour, pasta noodles, beef, and canned sardines. Overall these purchases contain more fats, refined sugar, and processed carbohydrates than farm or forest foods. Given this immediate disposal of cash it makes sense that reliance upon market foods is related to income group, with those in the lowest income group utilizing the lowest percentage and those in the upper income group utilizing the highest (Byron 2003). Those communities closer to San Borja and more integrated to the market also utilize more market foods (particularly purchased meats) and less forest foods (Byron 2003). Byron explains that this relationship is at least partly due to increased access to the market, greater social value put on market foods, and variation in community-related food acquisition techniques (i.e. Barbasco). Another possible explanation, not discussed by Byron, is the potential ecological degradation of the forest in communities closer to San Borja compared to those farther away, in which forest resources may be less plentiful.

Variation in purchase of market foods may also occur seasonally. Byron observed more market use in the wet/preharvest/lean part of the year in communities more integrated and closer to San Borja, with the greatest use occurring in the highest income group (Byron 2003). Thus,

the market may be a seasonal alternative to low availability of farm or forest resources for those that can afford it in that community. This hypothesis is further supported by lowest use of market foods in the same community during the part of the year with the highest rice stores and peak hunting. However, Byron's analysis shows that market use does not surpass agricultural and forest food use, regardless of level of integration (Byron 2003). This indicates that although market food may act as an important supplement, it is not necessarily an essential part of the diet.

Besides buying consumables, the Tsimane' also purchase a number of goods from the market. Many of the commonly used goods in the household all come from the market, including pots, water containers, and mosquito nets. Some purchased good may be useful for increasing agricultural and forest resource extraction, such as machetes, rifles, shotguns, and axes. Many Tsimane' have started to adopt and desire western luxury items including radios, shoes, clothing, watches, glasses, televisions, video cassette recorders, and even cell phones. Godoy found that with an increase in total expenditures there was a positive association with the share of expenditures towards luxuries (Godoy 2007). As the Tsimane' continue to be exposed to mainstream Bolivian and involve themselves more in the market system the desire and expenditures on luxury items will most likely continue to increase.

The effects of market integration on the health of the Tsimane' are not well understood. Currently market integration does not seem to affect their health. Byron suggests that the apparent lack of effect may be due to the modest levels of wealth and income or because wealth is neither being accumulated nor is it being saved and used in times of crisis (Byron 2003).

For now the Tsimane' still rely heavily on horticulture and the forest to provide them with what they need. However, this may change in the future, as people begin to allocate a

larger portion of their agricultural products to the market. Members of at least one community have been reported to sell rice early in the season to the point that they have to turn to alternative food sources or purchase more later on from the market (Byron 2003: 237). If these trends continues and the intensification of commodity agriculture does result in increased reliance on and desire for market-bought goods, it may ultimately remove the Tsimane's ability to sustain themselves independently of the market system (Piland 1991)

Conclusion:

This chapter has provided a brief overview of Tsimane' history, culture, ecology, subsistence, demography, and market involvement. The Tsimane' are a population of indigenous Amerindians who through their semi-nomadic lifestyle has managed to remain a distinct cultural group existing largely on the edges of the Bolivian market system. The Tsimane' subsist mainly through hunting, fishing, gathering, and some supplemental involvement in the market system. These subsistence bases and their level of involvement in each vary depending local ecology, community of residence, and distance to San Borja, Their variation in these traits has made them an ideal group in which to explore the effects of market integration and culture change.

Chapter 5: Leptin and Anthropometrics in the Tsimane'

Introduction:

This chapter examines the correlates of variation in leptin levels in adult Tsimane' males and females. I begin this chapter with a brief background on relevant Tsimane' anthropometric dimensions and briefly discuss the previous work on leptin conducted on the Tsimane'. I then review the data collection protocols and methods utilized for this study. Finally, I compare patterns of variation in Tsimane' leptin levels to those found in other populations. The objectives of this chapter are to examine the relationship between leptin and various measures of adiposity and to examine other potential predictors of leptin levels that determine variability in leptin independent of adiposity.

As discussed in chapter three, leptin is an indicator of energy status and changes dynamically in response to energy balance, acting to mediate some of the adaptive responses that attempt to maintain energy homeostasis, particularly in response to negative energy balance (Friedman 2002). Much of the research on leptin has been done in populations from the United States or Europe, thus providing little opportunity to examine the influence of ecological variability on leptin levels. The Tsimane' provide good contrast to those populations, as they are a population that has been previously characterized by growth stunting and lives largely a subsistence level lifestyle. This research not only helps to expand our knowledge of leptin and its variability but also provides a profile of leptin in a population that may be a better model in which to understand leptin's role in human evolution.

Background:

The Tsimane' are a population of approximately 8,000 Indigenous Amerindians from the department of Beni in lowland Bolivia. They have 50 villages along the Maniqu River with 30 additional villages near other rivers, with a mean village size of 93 individuals (Winking 2005, Gurven 2000). They are forager-hoticulturalist, relying mostly on subsistence swidden agriculture (i.e. slash and burn) and forest goods for their needs (Byron 2003). However, recently they have started to supplement their subsistence lifestyle with wage labor from cattle ranching and logging and through the sale of goods (e.g. *Jatata* thatch roofing). For a more extensive background on the Tsimane' please refer to chapter 2.

Anthropometrics in the Tsimane'

Although this dissertation is focused upon adults, anthropometric data and trends in both children and adults Tsimane' will be briefly reviewed here. The reason for this is that adult energetic states, particularly stature, but also body composition, can be considered an outcome of a long history of growth, occurring from conception to adulthood. Unfortunately no data currently exists on birth weights, gestation times, or pregnancy weight gains that would give insight into fetal growth and nutritional sufficiency. Additionally, previous work on leptin in the Tsimane' has only been done with children (Sharrock et al. 2008), making an understanding of their anthropometrics important background to help contextualize their leptin values.

Anthropometric data on children show the Tsimane' to be a population that is largely stunted compared to US or European norms (Foster et al. 2005; Byron 2003). However, compared to other Amerindians they are in the taller range (Godoy 2006). According to data

from Foster et al., boys appear to be around the 5th US percentile by 6 months, while girls are at the 50th percentile at 6 months and move to the 5th percentile sometime before the age of two (Foster et al 2005). It is unknown why the males show this pattern, but female growth restriction is probably due to the timing of weaning. Depending on age, the prevalence of stunting in Tsimane' children range from 55 to 31%, which decreases and compares more favorably to other Amerindian groups as they get older (Godoy 2005; Byron 2003). Unlike stunting, wasting is relative low (0-2%) and skinfolds as well as arm muscle development are adequate, although lower than US norms (Foster et al., 2005; Byron 2003). This pattern of high incidence of stunting and low incidence of wasting is indicative of chronic, mild to moderate malnutrition, a pattern consistent with other indigenous Amazonian groups (Godoy 2005, Foster 2005, Byron 2003). Although adult dietary data shows adequate caloric and protein intakes, due to the bulk of two of their main staples, manioc and plantains, children with their smaller gut capacities may not be able consume enough to get adequate nutrition from them (Godoy 2006; Godoy 2005; Berti and Leonard 1998). However, it is unknown how much children may offset this by consuming a larger proportion of non-bulky, more easily digested foods, such as rice. Even if caloric and protein sufficiency are met, dietary quality may still be lacking in other ways, such as micronutrient deficiencies (Nyberg 2009). High rates of iron deficiency anemia within Tsimane' children shows that such micronutrient deficiencies exist and may play a role in the stunted linear growth (Foster et al. 2005; McDade et al. 2005; Aiello et al. Unpublished). The Tsimane's stunting may also be due to early pathogen exposure, with hookworm infection (77% prevalence) being an endemic problem for the Tsimane' (Godoy 2008, McDade 2005a McDade 2005b, Tanner 2005).

Compared to other Amerindian groups Tsimane' adults are taller and heavier (Tanner 2005, Byron 2003). Tsimane' adults show anthropometric patterns similar to the children, with favorable to adequate short term or acute anthropometric measures (BMI, weight-for-height z-scores, arm muscle area, skinfolds), while stature is short compared to US standards, reflecting their childhood stunting (Byron 2003). This outcome reflects that most deficiency occurs during childhood and adult dietary intakes are more than adequate in both calories and protein with little evidence of acute nutritional deficiencies (Godoy 2006). In comparison to US norms, women have more favorable sum of skinfolds and arm muscle area than men, although women do show more seasonal variability in weight and body fat stores (Byron 2003). Byron found evidence of a weak but significant effect of season on measures of nutritional status, with lower measures being observed in the wet season, while Tanner failed to find any seasonal differences (Tanner 2005, Byron 2003).

Analyses of adult stature suggest that the patterns discussed above have been fairly consistent over time, at least since the 1920's. Godoy showed no evidence for a secular trend in stature from 1920 to 1980 in males or females (Godoy 2006). However, Tsimane' elders and some historical data suggest that the average heights were taller before 1920 (Godoy 2006).

Tsimane' and Leptin

Sharrock and colleagues (2008) reported data on anthropometric and leptin values in 487 Tsimane' children with ages ranging from 2 to 15 years. These data are comparable to the work presented here because they relied on the same blood spot analysis method, conducted in the same laboratory, at roughly the same time period. Sharrock's work found low leptin

concentrations overall, with values being lower compared to other groups using a blood spot to plasma conversion. In her oldest adolescent group (13-15 years), the females had a mean leptin of 3.51 ng/ml while the males had 0.45 ng/ml. Females showed a strong correlation between leptin and measures of adiposity (particularly in the older age groups), with fat mass or triceps skinfold having the strongest relationships. Male leptin values showed much weaker correlations with fatness, with measures of percent fat, fat mass, triceps skinfold, and suprailiac skinfold showing significant correlations in some, but not all age groups, while other measures of adiposity (BMI, biceps skinfold, subscapular skinfold) failed to show significant correlations in any of the age groups.

Data Collection and Methods:

Fieldwork was conducted for this study over a 15-month period from December 1st 2004 to March 18th 2006 in three villages. All of the methods discussed were approved by the Northwestern University Institutional Review Board (IRB #0732-016). Additionally, before working in the communities, approval was obtained from the *Gran Consejo Tsimane'*, the Tsimane's only formally recognized governing body, and by each community through community meetings discussing the project with each village as a whole. Following this each household in the community was visited and IRB-approved verbal consent scripts were used to recruit individuals into the study. Potential study participants included all adults 18 years and older.

Additionally, these data were supplemented by data from a larger research project, the Tsimane' Amazonian Panel Study (TAPS), originally began in 1999 by Dr. Ricardo Godoy

(Brandeis University) and Dr. William Leonard (Northwestern University). This larger study, now known as the Tsimane' Amazonian Panel Study (TAPS), is a longitudinal project encompassing 13 villages and over 260 households (www.tsimane.org). The main focus of this project has been on understanding the effects of market exposure on a variety of health indicators and markers of wellbeing.

Collection Rounds

Three rounds of data collection occurred in total (see table 5.1), with round one being conducted from December 2004 to February 2005 (Wet season), round two being collected from June to August 2005 (Dry season), and round three being collected from February to March 2006 (wet season). Laboratory analysis of blood spot samples occurred in July and August, 2006.

Three Tsimane' research assistants helped me to coordinate and collect data in the different locations. Two worked during collection rounds one and two, while only one worked during round three. Research assistants translated from Spanish to Tsimane' during interviews and training with surveys before rounds was used to ensure meaning was held constant across translators and their translations.

Sample Size

A total of 108 individuals participated in this research study, including 60 females and 48 males, with varying levels of participation from individuals in each of the rounds. The larger number of females resulted from a number of single women in the study as well as a number of husbands refusing to participate. Bloodspot data from the first round was excluded from this

analysis due to the samples being exposed to repeated freeze/thaw cycles during repeated power outages and people who only participated in round one were excluded from this analysis (n=16). The remaining 92 individuals (52 females and 40 males) from rounds two and three were further reduced to 67 (37 females and 30 males) to exclude individuals who did not have both leptin and anthropometrics from the same collection period. The largest degree of within-round non-participation was seen in bloodspot collection, largely due to the logistic limitations in collection (one or two days of collection in the morning in each village/round). A single set of cross-sectional data was compiled from those 67 individuals, excluding data from rounds in which females were pregnant. This data was excluded due to the established understanding that leptin levels are elevated during pregnancy (Henson and Castracane 2006) and that there are problems associated with obtaining accurate measures of fatness using skinfolds from pregnant women (Lederman 2005). Only one female was completely dropped from analysis due to her pregnancy in both collection periods, while 10 other women had alternative round data selected due to pregnancy. If usable data existed for single individuals from both the dry and wet season, the data from the dry season was preferentially used due to that being the larger data set. The end result was a set of cross sectional data consisting of 66 individuals, 36 females and 30 males. For the analysis focusing on leptin and reproductive status 10 cases of pregnancy were added back to the data set and treated as independent cases. Nine of the individuals are then represented twice in the data, as pregnant and non pregnant, and were also analyzed separately as paired samples.

Surveys

Baseline/Demographic Surveys

Interviews collected demographic data, including household size/composition, age, education, and basic markers of acculturation. Identification of age is problematic among older individuals as records have only recently started to be collected. However, my data was cross checked with data accumulated from TAPS in order to arrive at the best available estimate. Level of acculturation was assessed based upon knowledge of Spanish, reading, writing and mathematics (Godoy 2001). Interviews were also conducted with female participants to collect information on basic reproductive histories (e.g. age at menarche, parity) and to determine reproductive status (non-lactating, pregnant, lactating, and/or menstruating).

Market Integration/Economic Surveys

Market integration was measured by examining cash income, ownership of non-local material goods, utilization of forest and grown foods compared with the percentage of foods traded or bought, and distance from and frequency of travel to San Borja. Individual economic activities were measured for the past week and month. This involved assessing the number of times individuals were engaged in various forms of production or consumption. Household material wealth was accessed by adapting a household goods material wealth survey from the TAPS project. The adapted survey measured 21 common household items. These items were given monetary value in Bolivianos and three summary variables were computed: traditional wealth, commercial wealth, and animal wealth.

Anthropometry

Anthropometrics were taken to assess nutritional status (Gibson 2005). Data collection was done as a community event, with participants gathering at the project house or the school to be measured. All people who came were provided with punch and those who participated received cookies. Anthropometric measurements were collected following protocols presented in Lohman et al (1988). Height was measured to the nearest 1.0 mm using a portal stadiometer (Seca model #214), weight was measured clothed without shoes and socks to the nearest 0.1 kg on a Tanita scale (# Bf-681). Body fat percentage was also recorded using the Tanita's bioelectrical impedance analysis (BIA). A Lange skinfold caliper (Beta Technology Inc, US) was used to take skinfold measurements at the following four sites: triceps, biceps, suprailiac, and subscapular. Skinfold thickness was read to the nearest 0.5 mm. Body fat percentage was later calculated from the sum of four skinfolds and from triceps skinfold alone using the age and sex specific Durnin and Womersley formula (1974). Mid-arm, waist, and hip circumferences were all measured to the nearest mm using a fiberglass, self winding, flexible measuring tape. Where applicable all measurements were taken on the left side of the body. If measurements could not be made without clothing then 0.5 mm or 0.5 cm were subtracted to control for it. Anthropometric data from round II for Alta Gracia was taken from data from TAPS.

Arm muscle area was calculated using mid arm circumference and triceps skinfold, then corrected for sex specific bone diameter (Frisancho, 1990). Fat mass was calculated as percent body fat divided by 100 and multiplied by weight. Z-scores for height for age, weight for age, weight for height, and arm muscle area were all calculated using Frisancho 1990 reference data that was derived from NHANES I and II.

Bloodspot collection

Bloodspot samples have been collected previously in this population as part of a larger ongoing study (McDade et al. 2003), and this has proven to be a locally acceptable procedure with very low rates of refusal. Samples were collected primarily to be used for the analysis of the hormone leptin, an indicator of short and long-term energy status, with lower serum leptin levels indicating deficiency and higher levels reflecting energy surplus (Flier 1998).

The protocol is an adaptation of a commercially available ELISA kit developed for measuring leptin in plasma (Biosource CytoSets #CHC2284). Prior research has measured leptin in serum or plasma, but venipuncture is a relatively invasive procedure that is not likely to be acceptable to the Tsimané. In addition, the remote field conditions make it difficult, if not impossible, to centrifuge and freeze plasma. Dried blood spots provide a minimally-invasive alternative, as sampling is relatively easy and painless, and filter paper stabilizes analytes in whole blood (McDade et al. 2005; McDade et al. 2000). Validation of the blood spot leptin protocol has been published, and analysis of matched blood spot and plasma samples indicates a very high degree of correlation (Miller 2006, Pearson R=0.976). The biggest limitation of this method is that samples begin to degrade at room temperature after 3 days and during refrigeration at 14 days, thus samples should be stored in a freezer as soon as possible following collection.

Blood collection was all done in the morning between 9:30am and 12:30pm, in order to limit the effects of diurnal variation upon leptin. After wiping away the first drop of blood, a sample was collected in a cuvett for measuring anemia and subsequent blood drops were collected by placing at least one drop of free flowing capillary whole blood on standardized filter

paper (Whatman no.903, Middlesex, U.K.). Samples were allowed to dry for 4 hours and then returned to San Borja (within 24 hours) to be stored in a freezer. Due to unreliable electrical power the samples were then shipped on icepacks to a laboratory (Laboratorio de Investigación y Diagnóstico Veterinario, LIDIVET) in Santa Cruz where they were stored at -40°C until they could be hand carried on a flight back to the US and then stored at -20°C in the Laboratory for Human Biology Research (LHBR) until analysis. During the trip from Bolivia to the US samples were kept on ice with arrival temperatures of 1°C. All blood collection supplies were saturated in a bleach solution and later incinerated in a small pit following recommended field procedures for biohazardous waste disposal (Sharmanov 2000).

All bloodspot analyses were conducted in the Laboratory for Human Biology Research at Northwestern University. Leptin levels were measured using protocols developed in the lab (Miller et al. 2006). Twenty-two of the 30 male samples included in the data for this chapter fell below the lowest standard in the assay and estimated values were used. Variation across assays was monitored using low, medium, and high controls, with the inter-assay percent coefficients of variation were 10.3%, 7.1%, and 9.4%, respectively.

Statistics

Data was entered and statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) version 21.0. Variables were examined for normality using descriptive statistics (mean, median, mode), Shapiro-Wilk tests, and the examination of Normal Q-Q plots. Variables that deviated significantly from normal were log transformed. To more easily allow comparisons to other data bloodspot leptin values were transformed into plasma

values using the following equation derived from a set of bloodspot/serum samples: Plasma value = 2.12*Bloodspot – 0.05. Blood spot leptin was log transformed to normalize the data for statistical test, rather than the plasma transformed data due the presence of three negative values in the plasma transformed data. Independent T-tests and Mann-Whitney U (for non-normal data) were performed on descriptive statistics to examine male and female differences. Pearson correlation coefficients were calculated to describe the relationships between leptin and various measures of nutritional status in each sex. A Wilcoxon signed ranks test was a non-parametric test used on paired samples to test for statistical differences as an alternative to a paired t-test.

Regression analysis was used to determine significant predictors of leptin in addition to measures of nutritional status. Kruskal-Wallis H test was used to test for significant leptin differences between for woman of various reproductive states. Significance was set at a $p = 0.05$ level.

Results:

Table 5.2 show basic descriptive statistics for Tsimane' males and females. Using an independent T-test and a Mann-Whitney U-test, male and females show significant difference in all variables shown (including leptin) except age, BMI, waist circumference, hip circumference, and waist-hip ratio. In table 5.5 regression models 1 and 2 show that sex remains an important determinant of leptin levels when included in a model with fat mass, suggesting that the sex difference in leptin levels is not solely a product of differences in fatness.

In table 5.2 measures of adiposity for both males and females indicate adequate levels of nutritional status, with over 20% of males and females having body mass indexes over 25 kg/m^2.

The nutritional indices in Table 5.3 show that 11.11% of females and 43.33% of males are stunted. This large sex-based difference in stunting prevalence is most likely a product of sampling, as previous studies report comparable stunting rates between males and females (see Godoy 2005 for example). In females, other nutritional indices compare more favorably with US norms, reflecting what was also seen in the measures of adiposity. However, 10% of males have low (< 2 SD) arm muscle area z-scores compared to US norms. This greater deviation in male muscularity compared to female muscularity is consistent with previous work done in the Tsimane' (Byron 2003) and has been recognized in other populations that experience varying levels of malnutrition during growth (Stini 1972). This suggests that male muscularity is either less buffered from the environment or that females experience disproportionate levels of arm muscular development due to higher levels of physical labor compared to US populations (Black et al. 1977).

Table 5.2 reports plasma leptin value of 2.458 ng/mL in females and 0.261 ng/mL in males. These numbers are among some of the lowest observed, and are lower than adolescent Tsimane', Ache levels, and levels from the United States (Figure 4.1a and 4.1b). However, this comparison is made with some caution, as our levels are derived from a blood spot assay known to be sensitive to environmental conditions and have been mathematically transformed to plasma values, making comparisons potentially problematic (Miller et al. 2006). The Tsimane' adolescent comparison is less problematic as it was analyzed using the same methods, in the same laboratory. Indeed, the Tsimane adults are most similar to the Tsimane' adolescent values. With those methodological issues, the Tsimane' levels resemble Ache levels more so than US

values, despite body fat levels across all three adults groups being comparable. This suggests that some other factor besides fatness may be determining inter-population levels of variation.

Figures 4.2a and 4.2b show the relationship between Tsimane' leptin and fat mass for females and males. The female graph shows leptin levels are strongly associated with fat mass, while the male graph shows a more modest association. A regression model (not shown) testing for an interaction of sex and log fat mass did not show a significant interaction effect.

Table 5.4 presents the correlations between leptin levels and various measures of adiposity. For all measures of adiposity, except the subscapular skinfold, females show significant correlations with leptin levels. In women, the triceps skinfolds show the strongest relationship with leptin levels. Males show significant correlations with most measures as well, except biceps skinfold, BIA determined percent body fat (approaches significance), and body mass index. The biceps skinfolds are small and show little variability in males compared to other skinfolds, potentially explaining its non-significance. Bribiescas (2001) also found no relationship between leptin and percent body fat as determined by BIA or with BMI, potentially indicating that those measures lack sensitivity to adequately detect the weaker relationship between leptin and adiposity that exists in males, particularly those that do not represent populations where Tanita leg to leg BIA equations were derived.

Regression analysis, performed on males and females separately, using leptin (logged) as the dependent variable and fat mass (logged) as the independent variable were performed to test for significant effects of other dependant variables (results not shown). Variables that were not significant predictors of leptin include season of data collection (wet or dry), time of day the sample was collected, participants' village, age, health status (sick in the last two weeks),

acculturation index, measures of market integration, and reproductive status (i.e. lactating and menstrual status).

Additional analysis was carried out to more closely examine reproductive status and its relationship to leptin. For this analysis 10 cases were added back to the data set consisting of pregnant women. The pregnant women have a mean plasma leptin level of 4.682 ng/mL, nearly twice as much as non-menopausal woman (Figure 5.3). A Kruskal-Wallis H test revealed significant differences in leptin levels between the 5 reproductive states ($H(4) = 14.598$, $p = 0.006$), while no such difference existed between their measures of fat mass (Figure 5.4, $H(4) = 6.475$, $p = 0.166$). A Mann-Whitney Test U showed no significant differences between the Menopausal woman and the three groups of non-pregnant women ($U = 28.0$, $p = 0.070$). However, a significant difference was found between the pregnant women and three groups of non-menopausal women ($U = 60.0$, $p = 0.003$). This difference remains when I remove the 9 non-pregnant woman from the data set who are also match the individuals who are pregnant ($U = 48.0$, $p = 0.009$). In Figure 5.5, I examined only the 9 matched woman in their pregnant and non-pregnant state, who showed a significant difference in a Wilcoxon Signed Ranks Test ($Z = -2.547$, $p = 0.011$), while they show no significant different in body fat mass ($Z = -1.481$, $p = 0.139$). A regression analysis with leptin as the dependent variable and fatmass and a binomial pregnancy variable (pregnant yes/no) as the independent variables for the set of non-menopausal women showed pregnancy was a significant predictor when controlling for fat mass (Table 5.3, model 3 for fat mass and model 4 for fat mass and pregnant as predictors).

Discussion:

These results show that the Tsimane' are a population with adequate levels of current nutritional status, except for low arm muscularity in males, compared to US norms. However, they show signs of chronic early life nutritional inadequacy with stunting occurring in both males and females (Stein et al. 2010). These results are in agreement with the past studies on the Tsimane' reviewed in this paper and most likely indicate mild to moderate malnutrition during childhood. As discussed in the background, this malnutrition most likely reflects low quality diet (i.e. too much bulk or low micronutrient content) and childhood pathogen exposure.

Tsimane' leptin levels demonstrate clear sexual dimorphism, a consistent feature across the majority of populations, with females having much higher values (Wauters and Van Gaal 1999). Leptin in Tsimane' females has a strong relationship with measures of adiposity. Males have similar associations with skinfolds (and skinfold derived measurement) but show weak associations with other, potentially more problematic, measures of adiposity (BMI, BIA). Leptin in males also has a much flatter association with fatness at low levels of adiposity, similar to what has been seen in Filipino males (Kuzawa et al 2007, also see chapter 5).

Both males' and females' leptin levels are low compared to other populations and resemble what is seen in Tsimane' children and adolescents. This pattern of low leptin levels is consistent with other studies from subsistence level populations, including the Ache (Bribiescas 2001), Shuar (Lindgarde et al 2004), Buryat (Leonard et al. 2009), and Evenki (Leonard et al., unpublished, see data in Chapter 3). All of these populations have among the lowest leptin levels observed in humans. What leads to these low levels? Work by Lindgarde et al. show higher leptin levels in agriculturalist compared to hunter/gatherers, suggesting that some factor

contained within their broad lifestyle and ecological differences drive variation (2004). Animal models show similar results, with captive baboons having leptin levels three times higher than wild baboons, supporting the idea of a strong lifestyle related effect (Banks et al 2001). Mice models have suggested programming may play a role in leptin variability (Mainardi et al 2010). One study in humans has shown low early life fat intake to be associated with increased leptin later in life (Rolland-Cachera et al 2012).

Is this population variation in leptin levels functionally meaningful? There is evidence in Pima Indians that low leptin variability may predispose to later weight gain (Ravussin et al 1997), but it remains unclear what the significance of between population variation may be. Do these low levels play a role in the "thrifty phenotype" that has been hypothesized to predispose some population that experience poor environmental conditions early in life to obesity when as adults they live in obeseogenic environments (Bouchard et al 2007)? If so, one might predict rates of cardiovascular disease, obesity, or diabetes to greatly increase in the Tsimane' as they become market integrated and continue to transition away from traditional lifestyles.

This study did not find significant predictors of leptin levels among the majority of variables investigated outside of adiposity measures. Part of this lack of association may be due to the small sample size of this study which may lack the necessary power to detect significant differences for some of the variables tested. Some of these potential predictors may also have lacked the necessary precision, such as the sickness variable, which consisted only of a binomial yes/no measure of sickness for the past two weeks. Additionally there may be untested factors that could contribute to leptin variability. These include measures of energy balance, dietary factors, and various other lifestyle measures.

Reproductive status was a significant predictor of leptin while controlling for adiposity. Pregnant women were shown to have higher leptin levels than non-pregnant/non-menopausal women. This relationship has been well documented in westernized samples (Henson and Castracane 2006, Castracane 2006, Mukherjea et al 1999, Butte et al 1997). Bribiescas reported in his sample one pregnant Ache woman, with leptin levels elevated compared to the rest of his female samples (2001). In previous studies the increase during pregnancy is typically 2 to 4 fold, with differences in this study being a little under 3 fold (see Figure 5.5) (Linneman et al. 2000). Unfortunately due to the small size and imprecise nature of our pregnancy data more detailed analysis such as analyzing for trimester difference are not possible. This study does provides additional evidence that this pattern and proportion of increased levels during pregnancy holds true across populations. The exact function of this rise in leptin is unclear, although it has been suggested that a state of leptin resistance occurs to allow the accumulation of additional body fat during pregnancy (Augustine et al. 2008, Chehab et al. 1997), but it may also play important roles in energy regulation to the fetus or in fetal programming (Chehab et al. 1997).

Conclusion:

In conclusion, these findings contribute to the available data on leptin across various ecological conditions. Leptin levels in Tsimane' adults are low compared to other populations and show continuity from values previously obtained in Tsimane' adolescence by Sharrock et al. (2008). This study adds to the body of evidence that the observed patterns of leptin sexual dimorphism are maintained across populations. Additionally, it provides clear evidence for

pregnancy related increases in leptin levels similar to what is experience in other population in which it has been reported.

Chapter 6: Leptin in Filipino Adults

Introduction

This chapter presents data on leptin levels in adult Filipino males and females. I begin this chapter by reviewing the data collection protocols and methods utilized for this study. I then offer an interpretation of these findings in comparison to those of previous studies. The objectives of this chapter are to examine the relationship between leptin and various measures of adiposity and to examine other potential predictors of leptin levels that determine variability in leptin independent of adiposity

As discussed previously in Chapter 3, leptin is a hormone secreted primarily by adipocytes and is thought to play a role in energy regulation and to act as an energetic indicator to various physiological systems (e.g. reproduction and immune function). Since leptin's initial discovery in 1994 there has been a tremendous amount of research devoted to developing a better understanding of its functional significance in humans. Much of this research has been conducted in industrialized populations with relatively large adipose stores. These studies provide a rather limited scope of leptin's potential range of variation. Recent studies in relatively lean, less developed populations have shown much lower levels of leptin, as well as evidence that the association between leptin and adiposity may break down at low adiposity levels, particularly among males (Feeman AC, Yousafzat AK et al., 2002). This suggests that the relatively overweight industrialized populations that are the focus of most clinical research may provide an incomplete human model for the investigation of leptin's physiologic roles. Thus this

chapter examines leptin levels from a sample in the Philippines, a population that is in the midst of the nutritional transition.

Material and Methods:

Study Population

This study uses data from the Cebu Longitudinal Health and Nutrition Survey (CLHNS), an ongoing, community-based, prospective study in Cebu City, the second largest metropolitan area in the Philippines. The adults who are part of this analysis were originally enrolled in the study as part of birth cohort born in 1983-1984. These individuals have been tracked since that time using follow-up surveys. Previous research has shown large increases in rates of overweight and obesity in the recruited mothers since 1984 (Adair 2004). This increased prevalence occurred along-side rapid socioeconomic changes, during which the adults who were part of this study were growing up. Data for this analysis is part of a fifth wave of follow-up data collection that occurred in 2005 (see Adair et al. 2011 for a full discussion of CLHNS). This research was conducted under conditions of informed consent with human subjects' clearance from the Institutional Review Boards of the University of North Carolina, Chapel Hill, and Northwestern University.

Data Collection

Trained interviewers conducted interviews with participants in their homes. Body weight, height, waist circumference, hip circumference, and triceps, subscapula, and suprailiac skinfold thicknesses were measured to the nearest millimeter using standard anthropometric

techniques (Lohman et al., 1988). Body mass index was calculated as the ratio of weight (kg) to height squared (m^2). Body fat percentage was calculated using Siri's (1956) equation from density calculated from the sum of the three skinfolds with the age and sex-specific Durnin and Womersly formulas (1974). The Durnin and Womersly formula was developed in a large Scottish sample and has been used and validated in a greater number of populations than other formulas (Norgan 1995; Ulijaszek 1992; Shephard 1991), and it has been used in previous CLHNS studies to calculate body fat percentage (e.g. Kuzawa et al. 2012; Quinn et al. 2012; Gettler et al., 2011). It has been shown in some studies to be valid in Asian populations (Wang and Deurenberg 1996; De Waart et al. 1993) but has overestimated body fat in other studies (Eston et al. 1995). It also tends to overestimate fatness in very thin or malnourished individuals (Shephard 1991). Unfortunately no widely used formulas have been specifically validated in the Philippines. Fat mass was calculated as percentage body fat divided by 100 and multiplied by weight. Arm muscle area was calculated using midarm circumference and triceps skinfold, and then corrected for sex specific bone diameter (Frisancho, 1990). Z-scores for height for age, weight for age, weight for height, arm muscle area, BMI, percent body fat, and triceps skinfolds were all calculated using Frisancho 1990 reference data that was derived from NHANES I and II.

The equations discussed above were also used in the calculation of body fat percentage in pregnant women and a few caveats should be considered when interpreting this pregnancy data when using these methods. The first is that the prediction equation used to calculate body density (Durnin and Womersly 1974) and percent body fat (Siri 1956) were both based on non-pregnant women (Lederman 2005). Water content of tissues changes with pregnancy, potentially increasing skinfold thickness measurements and decreasing the density of lean tissue, both of

which would inflate body fat values calculated from equations derived from non-pregnant women (Lederman 2005; McCarthy et al. 2004; Paxton et al. 1998; Shephard 1991). Additionally, patterns of fat deposition across various skinfolds may be different in pregnant women, potentially adding errors depending on the sites used for skinfold measurements (Forsum et al. 1989; Taggart et al. 1967). In later stages of pregnancy both of these pregnancy-related changes are likely to cause increased error (Paxton et al. 1998). However, in a comparison of various prediction equations using skinfold measures to predict fatness in pregnant women, the Durnin and Womersly equation results were not significantly different from fat derived from hydrostatic weighing across three time points in pregnancy and was the only equation to have an acceptable ability to predict the hydrostatic body fat percentage across all three time points (Miller and Ballor 1989). With these caveats in mind and the lack of other suitable equations using the anthropometrics collected for this study, the Durnin and Womersly equation was utilized for the pregnant sample.

Blood collection was conducted approximately two months after the initial interviews and anthropometrics. For blood collection, participants were asked to fast overnight for 12 hours, and venipuncture blood samples were collected in-home by medical technicians the following morning between 5:40 am and 9:30 am using EDTA-coated tubes. After separation, samples were frozen and shipped on dry ice to Northwestern University for analysis. All samples remained frozen at −80°C until thawed for analysis.

Laboratory Analysis

Plasma leptin was measured using the Linco Human Leptin Elisa kit (cat. #EZHL-80SK). Two protocols were used to analyze samples. All samples were initially analyzed using the sensitive protocol. Any sample that exceeded the upper assay range (> 33.3 ng/ml) was rerun using the regular assay protocol which had a range that extended to 100 ng/ml. Three samples that exceeded that range were diluted and were reun using the regular assay protocol. A correction value of 0.847 was calculated from 36 samples ran on both the sensitive and regular protocols. Samples run on the regular protocol (n = 78) were multiplied by this correction to eliminate protocol bias. All samples were assayed in duplicate, and control samples were included with each assay to monitor between-assay variation. For the sensitive protocol, the inter-assay coefficient of variation was 20.1, 4.9, 3.5, and 2.0% for the low (~0.726 ng/ml), medium (~1.479 ng/ml), medium-high (~3.795 ng/ml), and high (~18.883 ng/ml) controls, respectively. For the regular protocol, the inter-assay coefficient of variation was 3.5 and 1.6% for the low (~4.626 ng/ml) and high (~18.828 ng/ml) controls, respectively.

Sample Selection

Overall 1775 plasma samples were available for analysis. Three were dropped due to insufficient sample for analysis. Twenty individuals were dropped because they were not part of the larger data set. Forty-six were dropped because they lacked other necessary data. One hundred individuals were analyzed separately because they were pregnant. The final data set was 1706 individuals, 912 males, 694 non-pregnant females, and 100 pregnant females. Twenty

four individuals (all male) had leptin values that could not be differentiated from zero and were coded as 0.01 ng/ml.

Statistical Analyses

All analyses were performed with version 11 of the Stata Statistical Package (College Station, TX). Leptin levels, triceps skinfold, subscapular skinfold, suprailiac skinfold, body fat percentage, and fat mass were all log transformed to normalize the data. Waist and hip circumference data were not available for many of the pregnant women and were not included (nor was waist/hip ratio) in any of the analyses involving pregnant women. T-tests were used to test for sex differences in descriptive continuous data, while Chi-square tests or Fisher's exact test were used for categorical variables. Pearson correlation coefficients were used to compare the association between various measures of adiposity and leptin. Beta coefficients were used to examine the expected change in leptin for each one unit change in the anthropometric variables. Models using interactions between sex and anthropometric measures or indices were used to examine the effect of sex on leptin. Kruskal-Wallis tests and t-tests was used to examine the effects of pregnancy status on leptin.

Previous Work: Leptin and Anthropometrics in Filipino Adolescents

Leptin levels in a small sample of participants (293 males and 303 females) from the larger birth cohort study have been previously analyzed when they were adolescents, ages 14-16 years (Kuzawa et al. 2007). The objectives of this study were to present leptin concentrations and anthropometric data as well as present maturational and lifestyle correlates. This age group

was examined specifically because they were lean and had not yet undergone any age-related increases in weight gain that were seen in older individuals (Adair 2004). The pertinent results of this study were as follows: Both sexes were lean, with relatively low rates of overweight and obesity (< 3%). Leptin levels were very low in comparison to other adolescent groups. Triceps skinfold was the strongest predictor of leptin in both sexes, while males showed more of the variation in leptin being explained by differences in skinfolds. After adjusting for triceps none of the other anthropometric measures tested remained significant in males, while subscapular skinfold, weight, and BMI all remained significant in females. Significant predictors occurring independent of triceps skinfold were urban residence in males and percent energy from dietary fat in females. The results concerning leptin and maturation will not be discussed here.

Results

Table 6.1 shows basic descriptive statistics for Filipino males, non-pregnant females, and pregnant females. Sex differences between males and non-pregnant females were tested using independent t-tests and showed significant differences in all variables except age. Chi-square tests showed no significant differences in the prevalence of overweight or obesity in males and females. Underweight (BMI < 18.5) was significantly different between the sexes, with prevalence's of 15% in males and 29% in females. Despite the high prevalence of underweight and BMI z-scores that are skewed below zero (Figure 6.1a), percent body fat values are not low, with average values of 15.8% in males and 31.7% in females. Additionally, Filipino percent body fat z-scores and triceps skinfold z-scores have values that compare favorably to US norms

(Table 6.2 and Figure 6.1b). These results demonstrate that BMI values are not giving a completely accurate view of adiposity in this population.

Table 6.2 provides nutritional indices using z-scores derived from Frisancho 1990 reference data. These data show high rates of stunting in both males (46.8%) and females (40.5%). For females the other z-score indices show that they compare well with US values. However, 12.9% of males are underweight and 15.2% of them have AMA z-scores of less than -2.0. Forty-seven percent of those individuals who are underweight also have low AMA z-scores. The high underweight values here for males are most likely a product of low muscle mass and the high degree of stunting seen, not because of low fatness, as indicated by the relatively low rates of wasting. This deviation in muscle mass in males but not females was also seen in chapter 4 in Tsimane' males and as noted there it has been recognized in other populations that experience varying levels of malnutrition during growth (Stini 1972). The fact that males show numerous indicators of nutritional stress that are significantly worse than female indicators suggests that Filipino males are either more stressed or less buffered from the environment (culturally or physiologically) compared to females.

Table 6.1 reports average leptin values of 3.55 ng/mL in males and 18.20 ng/mL in females. Figures 6.2a and b present these values in comparison to Filipino adolescents and to Ache and US values. Compared to all three populations Filipino adults have high standard deviations. Compared to the adolescents, adult values are much higher in both males and females. Unfortunately the original 2007 paper by Kuzawa et al. on Filipino adolescents did not provide percent fat or fat mass values so direct comparisons are difficult. However, other markers of adiposity (i.e. BMI and skinfolds) are smaller in the adolescents compared to the

adult data in this study, explaining at least part of the difference. Despite the Ache having slightly higher percent fat levels, the mean Filipino leptin levels are over 3 times higher. In males both leptin levels and body fat levels are less than US values; however females have both fatness and leptin concentration values that are only slightly below that seen in the US.

Table 6.1 also presents descriptive statistics on a sample (n = 100) of Filipino pregnant women. Compared to non-pregnant woman there are no significant differences except that leptin levels are significantly higher in pregnant woman.

Table 6.3 examines the strength of leptin's relationship to various anthropometrics and measures of adiposity. In males all measures are significantly associated with leptin, with fat mass having the highest correlation coefficient. In females all measures of adiposity except waist/hip ratio are significantly associated with leptin, with fat mass also having the highest correlation coefficient. Overall measures of adiposity explain more of the variation in leptin in males compared to females. In pregnant women all measures are significantly associated with leptin except height, with correlations being lower than what is seen in males or non-pregnant females. For pregnant women fat mass and weight both have the highest correlation coefficient. Table 6.4 presents regression slope coefficients to examine the impact that a change in anthropometric variables has on leptin. All variables, except waist/hip ratio in females, have a significant effect on leptin. Additionally this table provides p-values for models that include with the interaction between the composition measures and sex in the model, with all variables except body fat percent, waist/hip ratio, and height having significant interactions.

Leptin plotted against fat mass is presented in Figure 6.3 for males and females. These plots show males having a leptin distribution that is more skewed towards zero, with the majority

of the points falling below 5.0 ng/mL, while the female values are much more spread out across the distribution.

Figure 6.4 examines mean leptin values across various pregnancy states (nonparous, first to third trimesters, and post-partum). A Kruskal-Wallis H test showed significant differences between groups. A t-test comparing leptin (log transformed) between pregnant (all trimesters) and non-pregnant (parous and post-partum combined) showed significant differences, $t(792) = -4.16$, $p < 0.000$, Figure 6.5 presents mean fat mass across these same groups, with significant differences between groups (Kruskal-Wallis H Test). However, a t-test comparing fat mass (log transformed) between pregnant and non-pregnant showed no significant differences, $t(792) = -0.27$, $p = 0.788$.

Discussion

These results show that the Filipino adults are a population with high levels of stunting compared to US norms, indicating past nutritional inadequacy. This is not surprising considering by age 12 months over 35% of the children in CLHNS were stunted and 96 % of children who were stunted at 6 months remained stunted at 2 years (Adair and Guilkey 1997). In females other measures of nutritional status compare well to US norms, while males show low muscularity. BMI data indicates high levels of underweight, yet percent body fat z-scores indicate levels of adiposity that compare favorably to US norms. As mentioned in the methods section the Durnin and Womersly (1974) equation used to calculate body fat in this population has been found to overestimate values in some, but not all Asian populations, potentially adding to some of this discrepancy. However triceps skinfold z-scores in table 6.4 also compares favorably to US

norms further supporting the idea that BMI is misleading. This misclassification by BMI is indicative of its problematic nature as a measure, particularly cross-culturally where body proportions (Wells 2010, Leonard and Katzmarzyk 2010) or body composition (Fernandez et al 2003) may alter its relationship to adiposity. It has been observed that compared to Caucasians of the same body fat percentage Asian populations tend to have BMI measurements 3 to 4 units lower, although Filipinos were not included in the study (Deurenberg et al. 2002). It has been reported that a BMI, age, and sex equation developed in Dutch children when used in Filipino children predicted body fat levels 5.9% lower than they actually were; a pattern consistent with what this study is showing in adults (Liu et al. 2011). The most probable explanations for these discrepancies are because of difference in body builds (e.g. leg length, slenderness) or differences in muscularity, with the latter being most probable in males with their low arm muscle area z-scores (Deurenberg et al. 2002).

As seen in the cross-population data (chapter 3), Tsimane' leptin data (chapter 4), and consistent with other studies (Wauters and Van Gaal 1999), this Filipino sample shows clear sexual dimorphism in leptin levels. Males and females show strong associations between leptin and measures of adiposity, with fat mass being the best predictor. Compared to other populations Filipino adults have leptin levels comparable to US values and values much higher than those seen in traditional subsistence populations like the Ache. This is also reflected in Figures 3.3a and b, where compared to other populations Filipino adult values fall above the regression lines and females show more extreme deviation by falling above the 95% confidence intervals. This is particularly interesting as adolescent Filipino values are much lower than the adult values. This is partially explained by increased fatness in adults, but may also have to do with the obesogenic

diet consumed by this younger generation (Kelles and Adair 2009). Perhaps there is an interactive effect between no longer investing energy in growth and this recent obesogenic diet, creating a drastic increase in leptin concentrations in the adult phenotype.

The higher leptin levels in Filipino pregnant women have been seen in other studies. These studies have repeatedly shown leptin levels to be elevated in humans in response to pregnancy (Henson and Castracane 2006; 2000; Mukherjea et al. 1999; Butte et al. 1997) with increased levels estimated to be on the order of 2 to 4 fold (Linneman et al. 2000). It is interesting that even though a significant difference in leptin levels during pregnancy exists for this sample, it is much lower than the 2- to 4-fold increase seen in other populations. However, it should be kept in mind the standard deviations are high and we are looking at cross sectional data, not longitudinal data. Other studies also agree that levels peak in the second trimester or early third trimester and continue to be elevated (or slightly decrease) through the remainder of pregnancy, compared to pre-pregnancy (Yang 2005; Sattar et al. 1998; Sivan et al. 1998; Tamura et al. 1998; Hardie et al. 1997; Masuzaki et al. 1997). Following pregnancy levels rapidly decline (Henson 2000; Yura et al. 1998; Masuzaki et al. 1997) again mirroring what is seen in this cross-sectional sample.

The source of the elevation of leptin in pregnant women can result from a number of pathways: increased maternal adiposity, production by the placenta, hormone-induced modulation of leptin synthesis or secretion, decreased leptin clearance (Mouzon et al. 2006), or a combination of these factors (Kafulafula et al. 2002). Some evidence suggests that the placenta may indeed be a major source of leptin during pregnancy, particularly given that levels rise before maternal adipose stores increase significantly in the first trimester (Highman et al. 1998).

Additionally, It has been reported using *in vitro* perfusion studies that 95% to 98.4% of placental leptin goes into maternal circulation (Lepercq et al. 2001, Linneman et al. 2000), estimating that the placenta may contribute to 14-15% of maternal plasma levels (Linneman et al. 2001, Linneman et al. 2000).

The lower correlation coefficients and the fact that weight and fat mass have the same relationship with the same strength ($r = 0.47$) may point to the potential problems associated with using the Durnin and Womersly equation to calculate fatness in pregnant women that was discussed in the methods section. The fact that the variables reliant on weight in their calculation (fat mass and BMI) have much higher coefficients compared to weight independent factors (percent body fat and the skinfolds) provides further evidence of this. This makes it hard to know if we are picking up real differences in the strength of the relationship between fatness and leptin in pregnant compared to non-pregnant women or if it is being driven by methodological issues.

The function of the increased leptin levels during pregnancy remains an open question. Chehab et al. (1997) have suggested that it may play an important role in maternal energy regulation to the fetus, fetal growth and development, or fetal programming. The non-satiety inducing increase suggests a state of leptin resistance (Augustine et al. 2008; Chehab et al. 1997). In mice increases in leptin levels are thought to be due to increases in the soluble leptin receptor (OB-Re), which may prevent leptin clearance from the kidneys (Reitman et al. 2001). It is not known if this binding effects leptin availability for signaling, but if it does it would be one possible explanation for the leptin resistance associated with pregnancy (Reitman et al. 2001; Gavrilova et al. 1997). However, pregnant human women either fail to show increased soluble

leptin receptors compared to non-pregnant women (Krizova et al. 2004) or show only a modest significant increase, in comparison to the increase in free (non-bound) leptin levels that is seen (Teppa et al. 2000; Lewandowski et al. 1999).

Conclusion

In conclusion, these findings from the Philippines, a population that has recently started to experience the nutrition transition, contributes to the available data on leptin across various ecological conditions. Leptin levels in Filipino adults are comparable to US values in stark contrast to those obtained from a sample from this cohort when they were adolescents (Kuzawa et al. 2007). This study adds to the body of evidence that the observed patterns of leptin sexual dimorphism are maintained across populations. It further demonstrates the problematic nature of BMI as a measure of adiposity and the lack of good field fatness measures in pregnant woman. Additionally, it provides much needed population diversity concerning data on leptin in pregnant women.

Chapter 7: Discussion and Conclusion

Introduction:

Much of our current understanding of human biology is from information extrapolated from a very select sample, mainly those people living in Western, industrialized countries. Such populations have recently been categories as WEIRD (Western, Educated, Industrialized, Rich, and Democratic) and have been critiqued on the grounds that they provide a very narrow and potentially anomalous sampling of human biology (Henrich et al. 2010; Lock and Kaufert 2001). Knowledge gained from such populations is reified in the supposed "norms" that are displayed as reference values such as those seen in assay kits. The assay booklet that came with the kit (Linco Cat. # EZHL-80SK) used to analyze some of this dissertation's data provides the following information in a section labeled "Normal Range" (p. 20, section XIV, dated 12/28/07):

> "Mean Serum Leptin levels from normal lean individuals
> (BMI ranges 18-25):
> Lean Men 3.8 ± 1.8 ng/mL
> Lean Women 7.4 ± 3.7 ng/mL"

This section provides no context for these numbers; the reader cannot discern if these are US values or if there are any known levels of variation within or across populations. As far as one knows, these values are indeed normal for all humans. Citations provided in this section reveal that these numbers come from a small US sample (n = 56; Ma et al. 1996).

This dissertation has taken a much broader perspective and challenged this view of normal. In reviewing the cross-population data on leptin I have contextualized values such as these, providing a review of the available data on leptin and showing more of the range of variability in leptin concentrations seen across a variety of contexts. Additionally, by examining

leptin in the Tsimane' and the Philippines this study has taken a closer look at leptin and its relationship to anthropometrics in two populations that occupy very different socio-cultural and ecological environments. The Tsimane' are a lowland Bolivian population of hunter-gatherers in the early stages of transitioning into the market system (Byron 2003), while the Philippines are a population further along in experiencing the nutrition transition, with shifts in eating patterns (Adair and Popkin 2005) and the associated increases in overweight and obesity (Adair 2004). This final chapter will provide a summary of the main findings of this dissertation, discuss their significance, note their limitations, and finally make recommendations for future studies.

Background Research Results:

Leptin is secreted primarily by white adipose tissue and leptin production is correlated with the amount of adiposity (Klein et al. 1996). Although initially portrayed as an anti-obesity hormone, it is more recently seen as primarily an anti-starvation hormone, with levels decreasing in response to energy restriction and potentially acting as an energetic signal of deficiency to other aspects of human physiology (Flier 1998).

Much of chapter three was devoted to reviewing the background literature on leptin and factors relating to its variability. Leptin shows diurnal variation, most likely tied to meal timing (Schoeller 1997), with peak levels occurring between one to four a.m. (Himms-Hagen 1999, Licinio et al. 1998) and lowest levels occurring around noon to mid-afternoon (Sinha 1996). Sexual dimorphism in leptin levels has consistently been seen across studies, with females having leptin levels two to three times higher than males (Wauters and Van Gaal 1999). In the majority of studies leptin is seen to have a positive relationship with levels of adiposity, to

decrease during periods of negative energy balance, and to increase during times of positive energy balance (Skurk et al. 2007, Chan et al. 2003, Chin-Chance 2000). Studies examining physical activity and leptin levels show mixed results, with many of the studies having methodological issues (i.e. failing to control for energy balance). Fat and protein levels show little effect upon leptin, while carbohydrates intake may have a positive relationship with leptin. Cold exposure, sympathetic nervous system activation, and increased catecholamines are all associated with decreased leptin levels. Increased stress, glucocorticoids, and various cytokines are all associated with increased leptin levels. Studies on hypoxia, alcohol, and smoking show conflicting results and all require further study to clarify their relationship with leptin.

Chapter 3 concludes with a broad analysis of cross-population data on leptin in attempt to capture the range of leptin variability. To gain a better understanding of broad, cross-population leptin variation, data was compiled from 25 populations for males and 37 populations for females. Across populations females showed higher leptin values and a greater range of variability compared to values in males. Fat mass was a better predictor of leptin levels than percentage of body fat in both males and females. Although subsistence-level populations do tend to have low values, there is a fair amount of variability seen in non-subsistence level population, with many showing deviations below the sex-specific, cross-population regression line.

Leptin in the Tsimane':

The Tsimane' showed low leptin levels in both males and females. When compared to the populations presented in chapter 3, Tsimane' males have the lowest leptin levels seen in

males and Tsimane' females have the second lowest levels (only the Evenki, reindeer herders of Siberia, have lower levels). These low levels are particularly interesting in that they are low when compared to populations with similar levels of adiposity. However, as mentioned in chapter 5 this comparison may be problematic as the Tsimane' leptin levels were measured using bloodspots, resulting in challenges associated with the conversion to plasma values. The Tsimane' adult levels were closer to the adolescent values then they were to other adult populations' values, showing fairly low levels of age-related variability. However, interpretations based on this data should be cautious as the data used is from cross-sectional age groups, not longitudinal tracking. Tsimane' females have much higher levels of leptin than males, a pattern seen in the majority of other populations.

Leptin in the Philippines:

The Filipino adults showed moderate to high leptin levels. Leptin levels in Filipino females are much higher than in males, consistent with the pattern seen in other populations. In comparison to the other populations presented in chapter 3 Tsimane' males have leptin levels that are above the sex-specific, cross-population regression line, but within the 95% confidence intervals (see figure 3.3a, population 13), while females have leptin levels well outside of the 95% confidence intervals (see figure 3.3b, population 22). In comparison, adolescent leptin levels are well below adult values. This increase in adult values is in direct contrast to what was observed in Tsimane' adult and adolescent leptin levels, where they were relatively similar. Direct comparisons between these populations are difficult but perhaps it has something to do with the dietary and lifestyle transitions that have occurred in the Philippines.

Leptin in Pregnancy:

Previous studies on leptin levels during pregnancy have shown levels to increase during the second and third trimester. However, very few studies have examined leptin levels during pregnancy in a non-western context. In the Ache, Bribiescas reported on one pregnant woman who had elevated leptin levels compared to the non-pregnant Ache women in his sample (2001). In Chapter 5 Tsimane', pregnant women were shown to have higher leptin levels compared to the non-pregnant sample of women and to have higher levels compared to their leptin levels during their non-pregnant state. The analysis in Chapter 6 of Filipino pregnant women showed they also had higher levels of leptin compared to the non-pregnant sample of women. This state of increased leptin levels during pregnancy seems to be a characteristic that holds true across all populations in which it has been measured and may be a general characteristic of leptin physiology in humans.

Limitations:

Chapter three's examination of cross-population data contains a number of limitations. Techniques to measure body composition, timing of blood collections, and specific study methodologies vary across studies and may be an uncontrolled confounder in our understanding of cross-population variability. Additionally the leptin values being compared across populations are the mean, a measure that is sensitive to the distribution of scores and thus does not capture all aspects of a population's leptin levels that may be meaningful for comparison (Williams 1991).

The population-specific analyses in Chapters 5 and 6 also contain a number of limitations. As noted previously, the leptin data in chapter 5 is derived from bloodspots, using a protocol that has only been used in one other study and has limitations due to low leptin stability (Miller et al. 2006). Additionally the Durnin and Womersly equations used to derive body fat and fat mass from skinfolds were developed using a Scottish population and, although it may be more widely used and validated than other such equations, they may add some degree of error when used in the Tsimane' or Filipino samples. This is may be even more problematic when it is applied to pregnant individuals.

One of the limitations of this dissertation in the examination of leptin during pregnancy is the cross sectional nature of the research design. Fortunately a small sample (n=9) from the Tsimane' provided a limited longitudinal snapshot by allowing comparisons of leptin levels from the same women in the pregnant and non-pregnant state. However, the small sample prevents more detailed levels of analysis (e.g. variability by pregnancy trimester).

Recommendations:

One of the major barriers in compiling the cross-population data was that many studies that measured leptin collected only BMI data rather than more detailed and accurate body composition measurements. This is important considering leptin's direct relationship with adiposity and as discussed in previous chapters the problems of BMI being an inadequate measure of fatness, particularly when used across a variety of populations, that may be different in a number of key factors, including their limb proportions or muscularity. Thus, it is recommended that whenever possible more direct measure of body fatness should be collected.

Field collections make this difficult, particularly on large scale studies with multiple field researchers collecting data, but at the very least skinfold or bio-electrical impedance measurements should be collected.

Once a measure of adiposity is obtained, it must still be decided what measure of adiposity if the most appropriate to use in analysis, with fat mass or percentage body fat being the two that seem most appropriate. Chapter 2 discusses the benefits and problems with each of these and ultimately concludes both should be explored in analysis. However, in the analyses conducted within this dissertation fat mass consistently gives higher correlation coefficients, although the difference tends to be fairly minor. Given the limitations of population specific body fat equations discussed above, examining raw skinfold measures may also be advised.

Analyses on leptin should be conducted separately on males and females. Chapter 2 offers a fairly lengthy discussion of sexual dimorphism in leptin. This discussion concludes that a sex difference does exist, with females having levels 2 to 3 times higher and that although body composition differences explain part of this, there may be other reasons as well. Additionally, the cross population data shows that these differences persist across a variety of populations. Therefore, at this time it would be prudent to analyze leptin data from males and females separately.

One of the issues with the Tsimane' data collection was related to the stability of the leptin assay used (Miller et al. 2006). Even more problematic is the fact that the kit components used in that assay are no longer available. Therefore, a more stable leptin blood spot assay, using readily available antibodies or kits, should be developed.

Future Studies

This dissertation has examined variability in leptin levels across populations, but it is still unknown what drives this variability and what, if any, its importance may be. Clearly a large part of the variability can be explained by differences in adiposity levels, but, as shown by the cross-population work in chapter 3, a fair amount of variability exists in populations that have similar body fat levels.

Finally, the majority of the studies involving leptin have been done in very limited populations, mostly WEIRD populations. More work, in a wider variety of populations, needs to be done linking leptin and appetite, examining variables that influence leptin levels, as well as examining the consequences of low or high leptin levels for a specific body composition. We know cross population variability in leptin exist, with much variability occurring at similar adiposity level but we know very little about the functional significance or consequences of that variation.

Conclusions

One of the strengths and benefits of taking an anthropological approach comes from the understanding that context, whether cultural or ecological, impacts biology. Since Boas' work in the early 1900s this has been a focus of anthropological research and has received increased attention as methods within biological anthropology have become more sophisticated. This study demonstrates the importance of taking this anthropological perspective by providing an in-depth examination of the considerable cross-cultural variability in a recently indentified hormone, leptin. These results make it clear that labeling leptin levels derived from a small

United States sample as an uncontextualized “normal” (as the assay kit previously mentioned does) is problematic and an effort should be made to avoid the assumption inherent in such labeling.

Figures

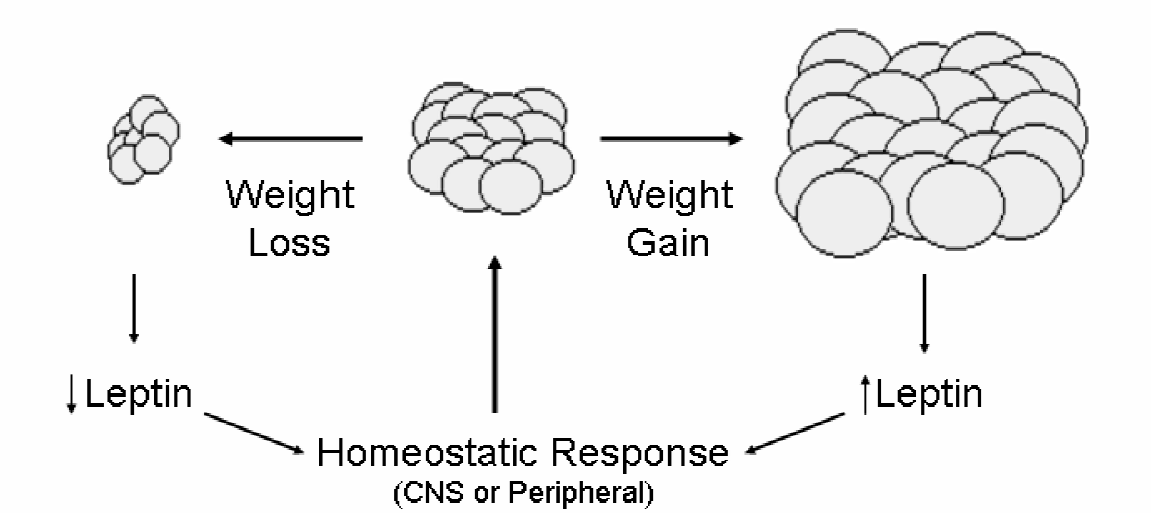

Figure 2.1 Traditional view of leptin's response to weight (i.e. adipocyte) gain or loss (adapted from Friedman 2002). In humans the responsiveness to weight gain is thought to be blunted compared to the response to weight loss.

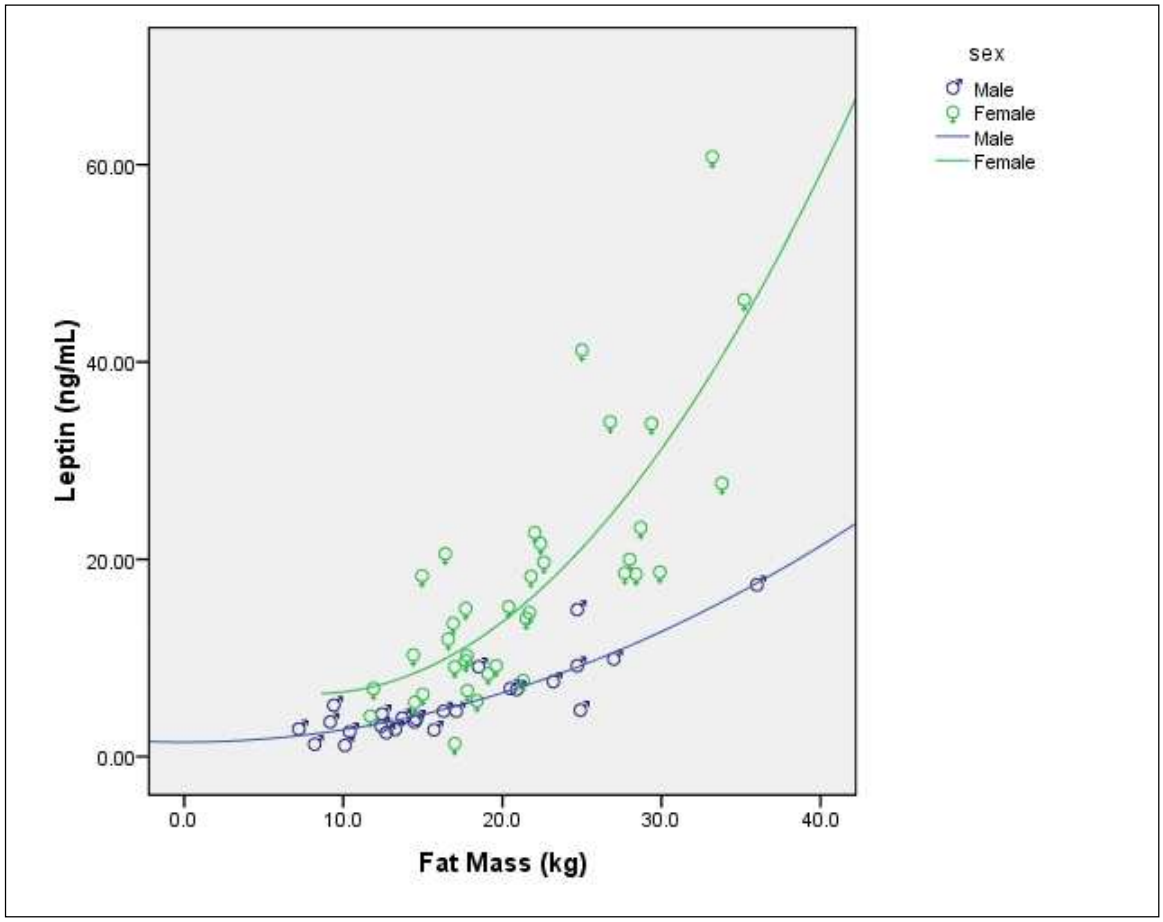

Figure 3.2: Cross-population data on leptin (ng/ml) plotted against fat mass (kg) and stratified by sex. Quadratic regression lines (Male $R^2 = 0.778$, Female $R^2 = 0.629$)

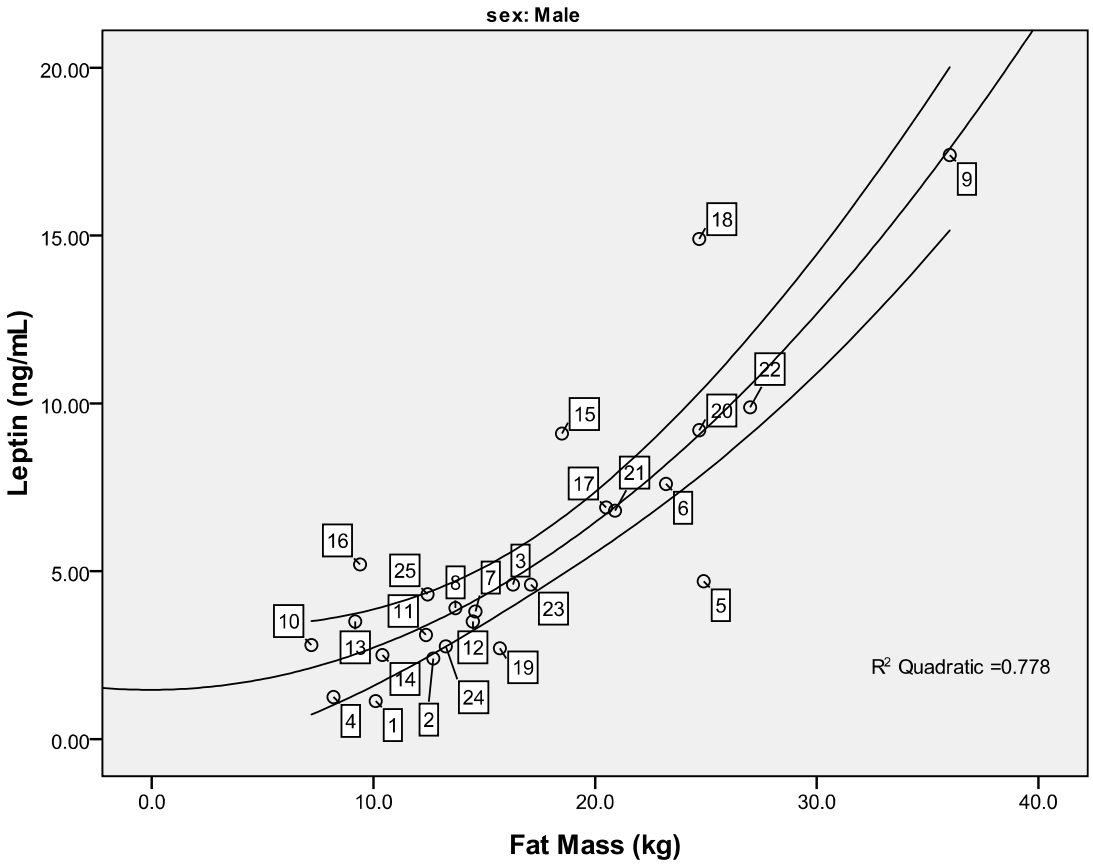

Figure 3.3a: Cross-population (males only) data on leptin (ng/ml) plotted against fat mass (kg). Quadratic regression lines ($R^2 = 0.778$) with 95% confidence intervals

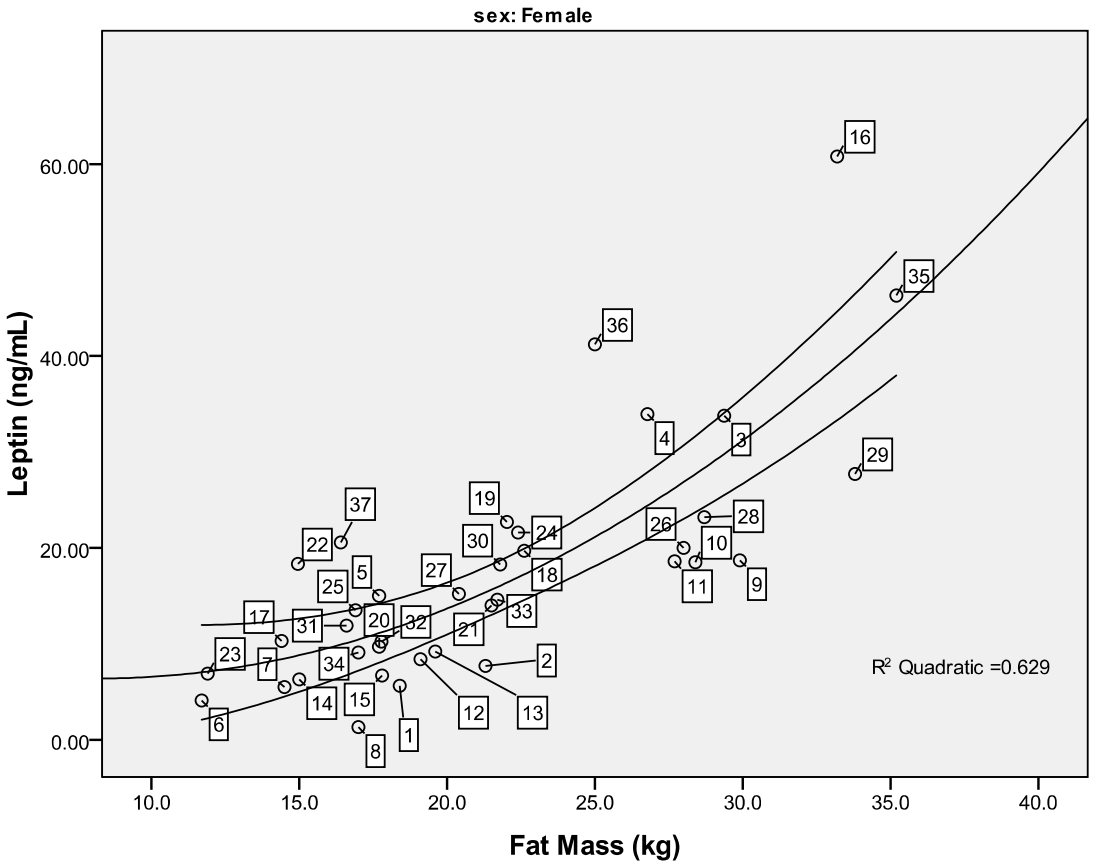

Figure 3.3b: Cross-population (females only) data on leptin (ng/ml) plotted against fat mass (kg). Quadratic regression lines ($R^2 = 0.629$) with 95% confidence intervals.

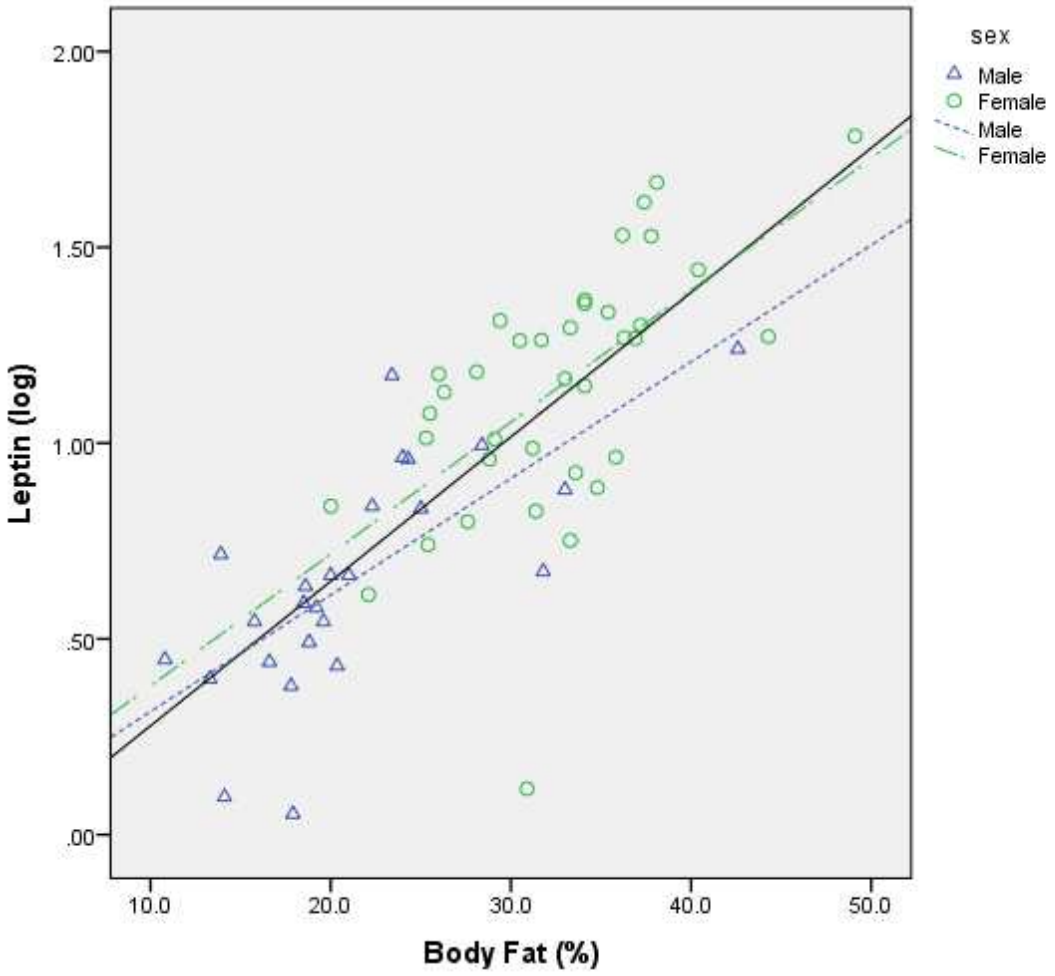

Figure 3.4a: Cross population data on leptin (log) plotted against percent body fat. R^2 for total = 0.625 (solid line). R^2 for males = 0.508. R^2 for females = 0.375.

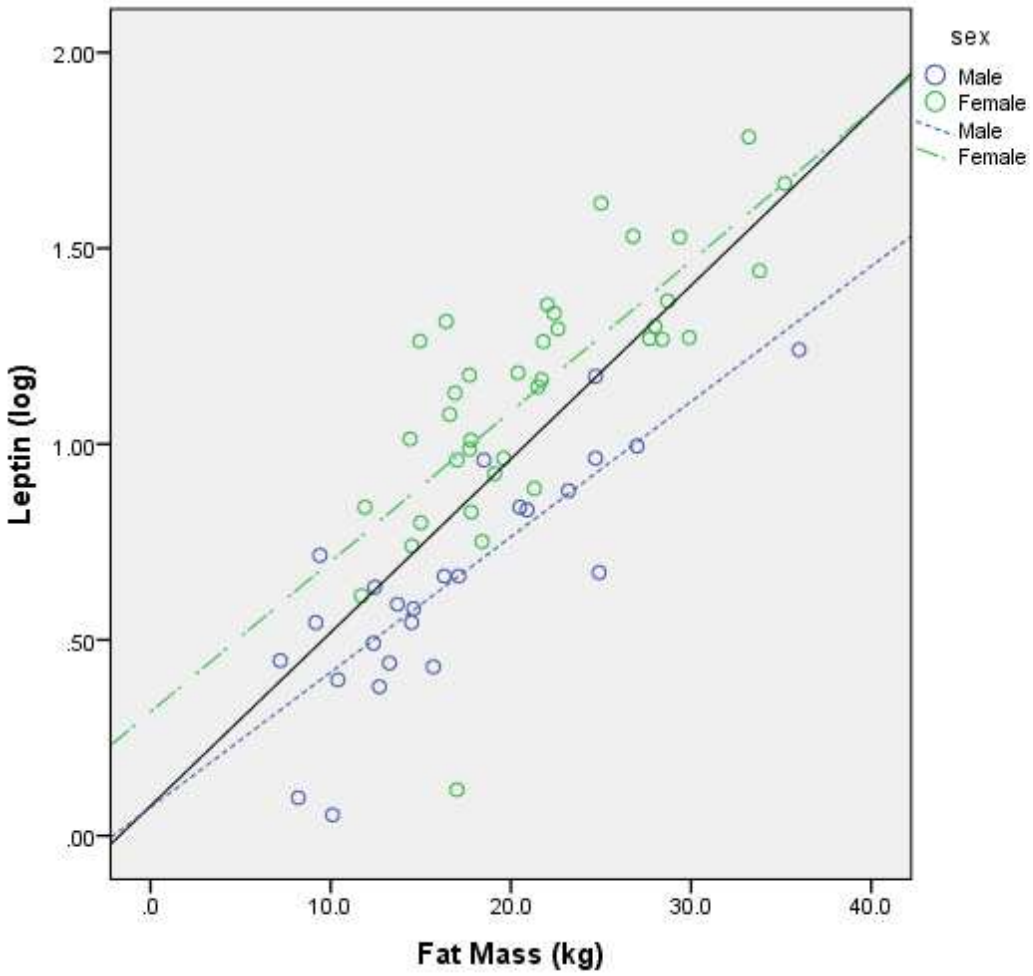

Figure 3.4b: Cross population data on logleptin plotted against percent body fat. R^2 for total = 0.609 (solid line). R^2 for males = 0.693. R^2 for females = 0.542.

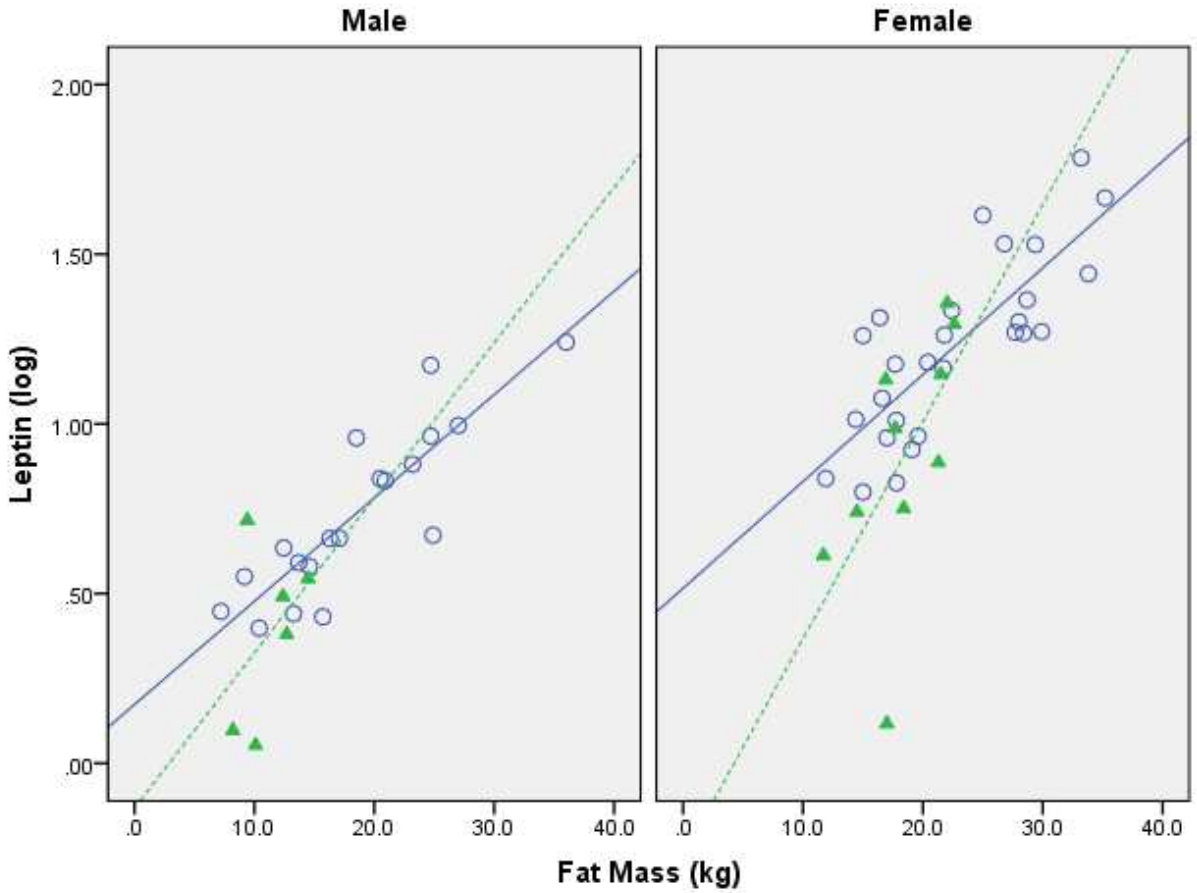

Figure 3.5: Leptin (log) plotted against fat mass (kg) and stratified by lifestyle for males (n = 25) and females (n = 37). Circle markers are populations that are urban or market integrated and triangle markers are populations that are rural or subsistence. Males: urban/market $R^2 = 0.754$, rural/subsistence $R^2 = 0.172$. Females: urban/market $R^2 = 0.636$, rural/subsistence $R^2 = 0.379$

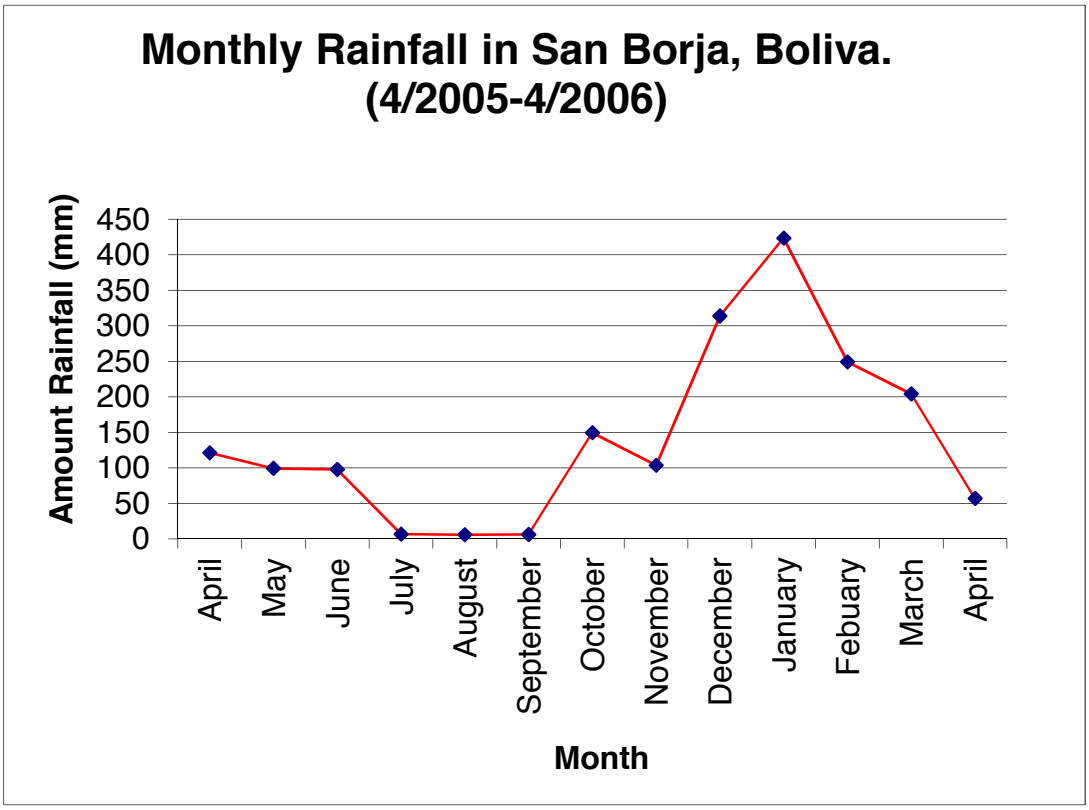

Figure 4.1: Monthly Rainfall in San Borja from April 2005 to April 2006

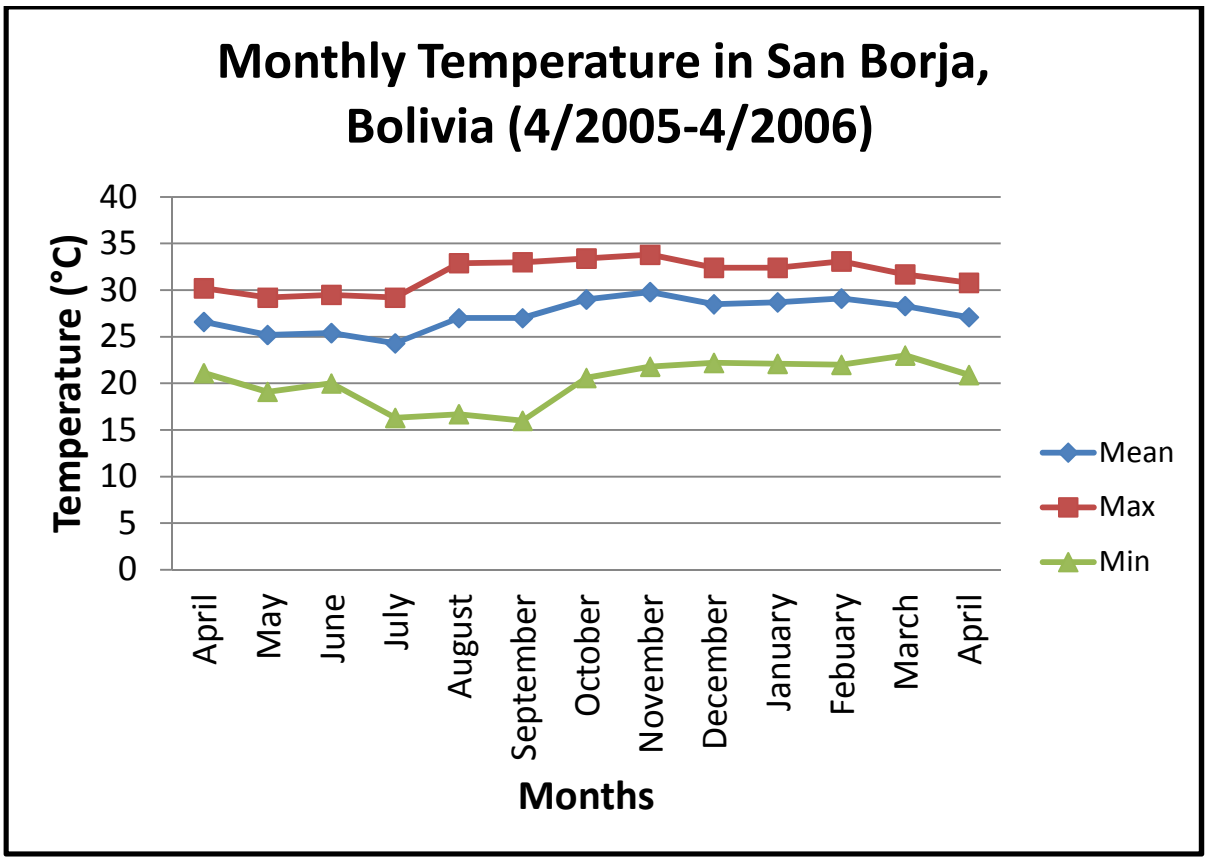

Figure 4.2: Monthly Temperature in San Borja from April 2005 to April 2006

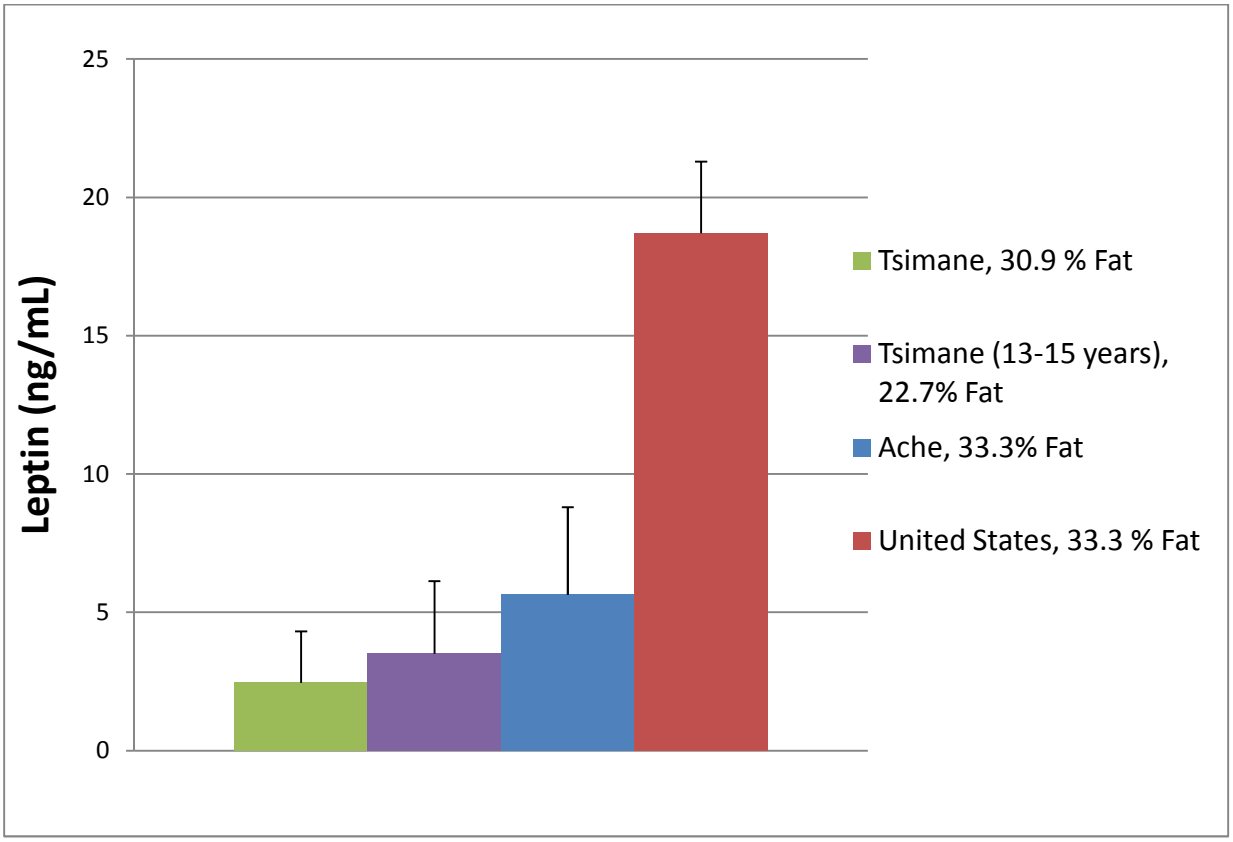

Figure 5.1a, Mean leptin levels for females in three different populations. Tsimane' data is plasma values transformed from bloodspot data (Plasma = 2.12*BS-0.05). Population labels include mean percent body fat values. Error bars on Tsimane' and Ache data represent standard deviation, on United states data they represent standard error. Tsimane' (13-15 years) data from Sharrock et al 2008, Ache data from Bribiescas, 2001, United States data from Kennedy et al, 1997.

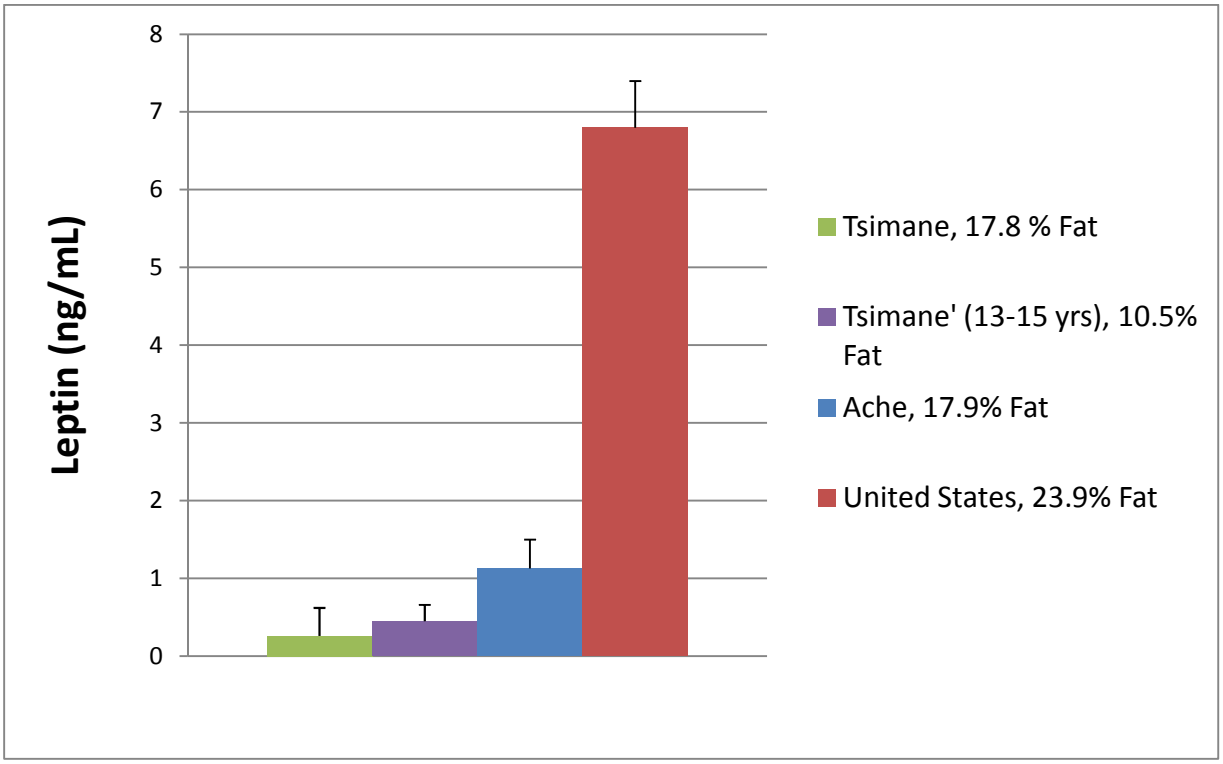

Figure 5.1b, Mean leptin levels for males in three different populations. Tsimane' data is plasma values transformed from bloodspot data (Plasma = 2.12*BS-0.05). Population labels include mean percent body fat values. Error bars on Tsimane' and Ache data represent standard deviation, on United states data they represent standard error. Tsimane' (13-15 years) data from Sharrock et al 2008, Ache data from Bribiescas, 2001, United States data from Kennedy et al, 1997.

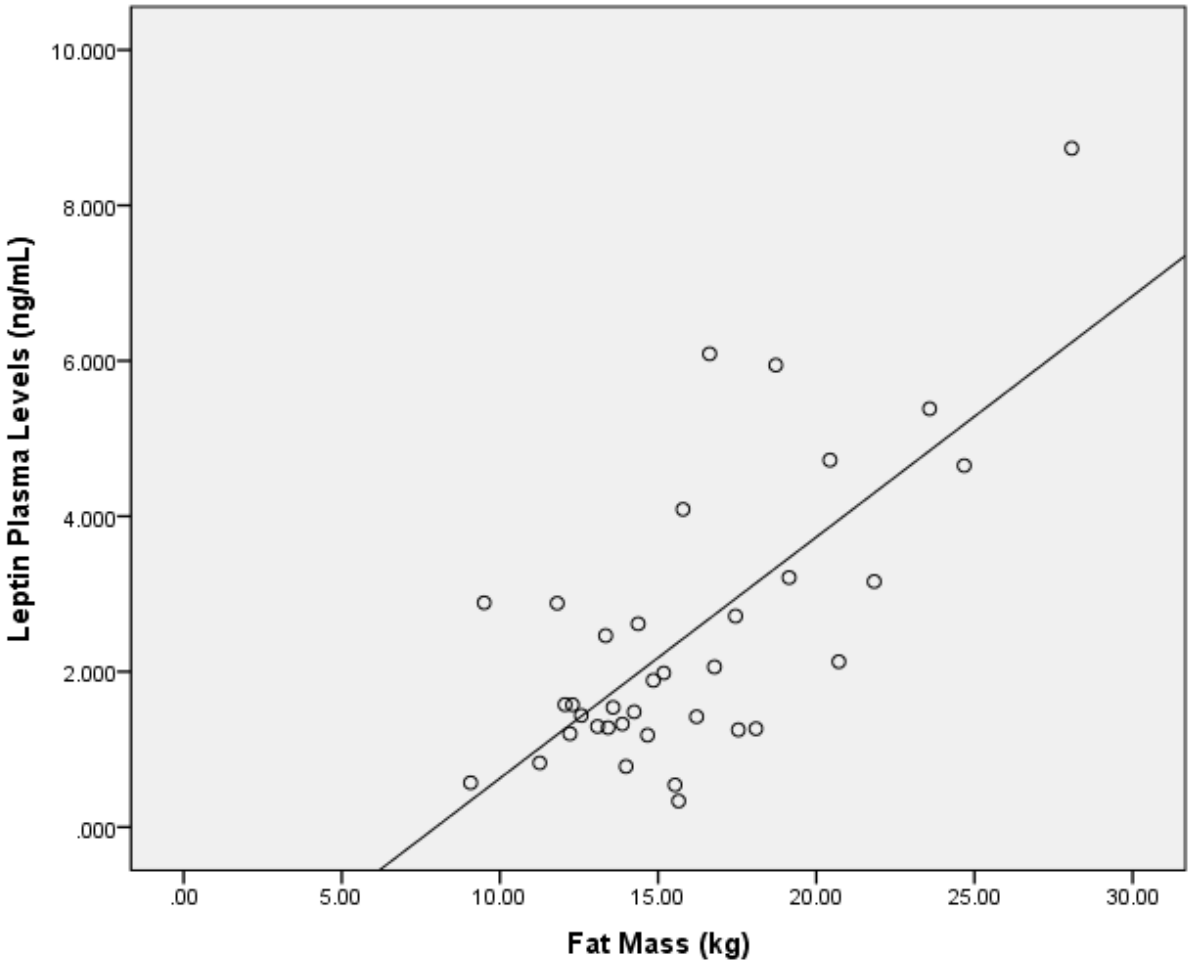

Figure 5.2a. Relationship between leptin plasma levels (ng/mL, calculated) and fat mass (kg, derived from triceps skinfolds) for Tsimane' females (Leptin = 0.32*Fat Mass – 2.47, $r^2 = 0.488$, $p < 0.001$).

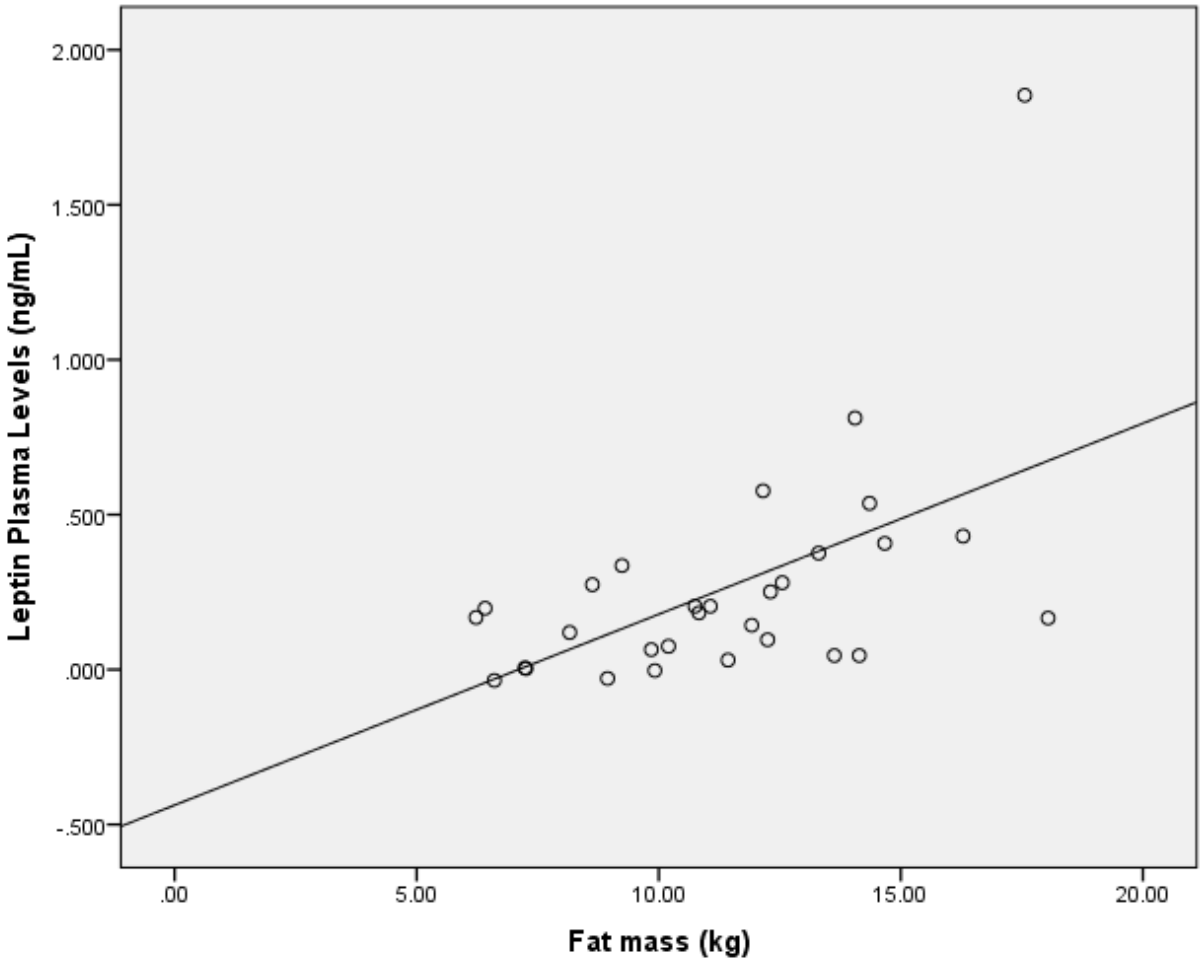

Figure 5.2b. Relationship between leptin plasma levels (ng/mL, calculated) and fat mass (kg, derived from triceps skinfolds) for Tsimane' males. (Leptin = 0.06*Fat Mass - 0.44, $r^2 = 0.299$, $p < 0.01$)

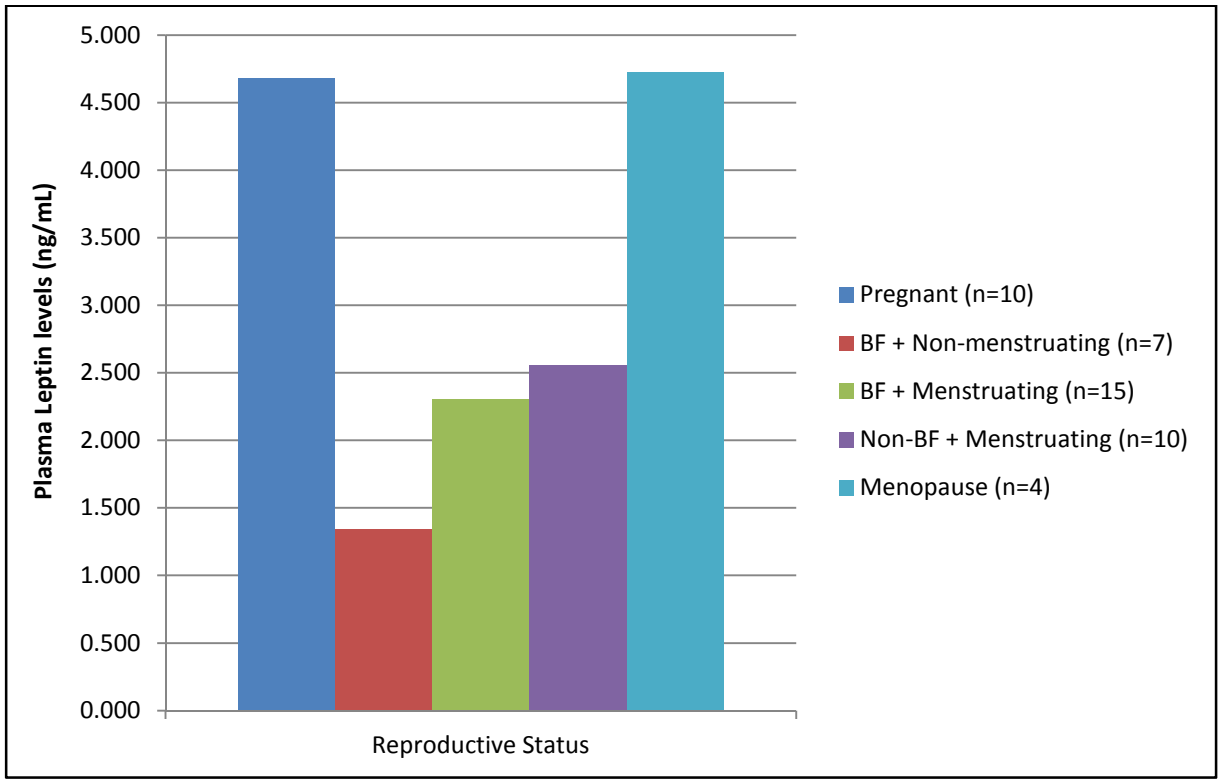

Figure 5.3. Mean plasma leptin levels across various reproductive states (n = 46). Plasma values are transformed from bloodspot data (Plasma = 2.12*BS-0.05). Pregnant women included here for comparison to samples included the majority of the analysis in this chapter. Kruskal-Wallis H test ($H(4) = 14.598$, $p = 0.006$).

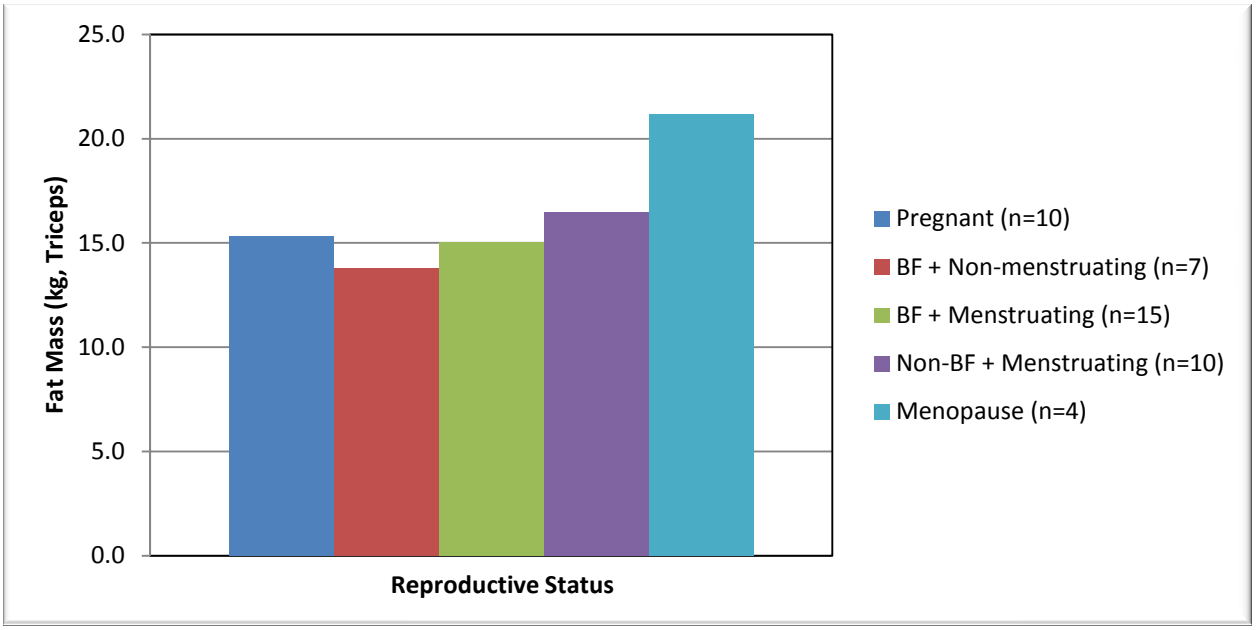

Figure 5.4. Mean Fat mass (kg, Triceps) across various reproductive states (n = 46). Kruskal-Wallis H test (H(4) = 6.475, p = 0.166).

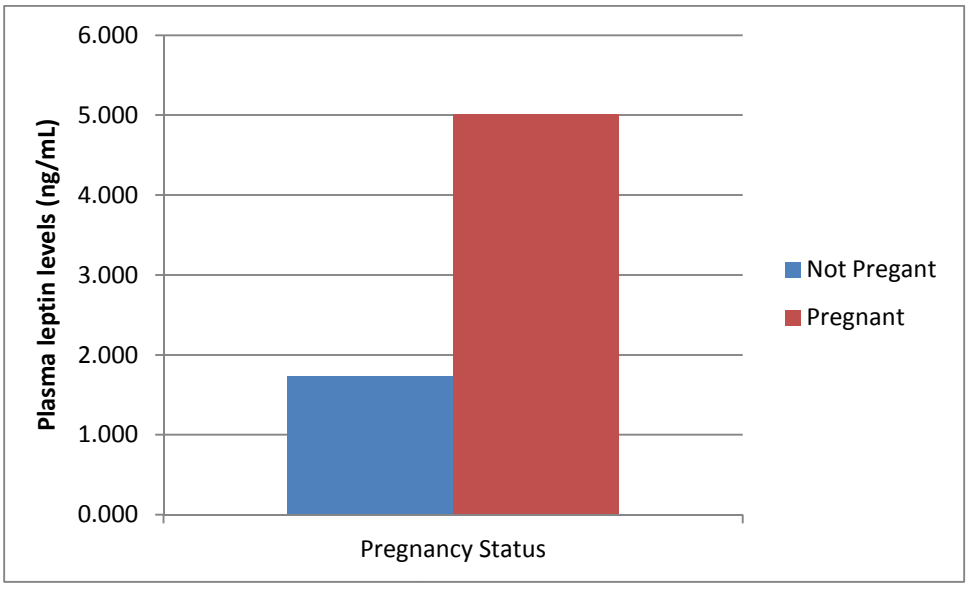

Figure 5.5. Mean plasma leptin levels in individuals by pregnancy status (n = 9). Plasma values are transformed from bloodspot data (Plasma = 2.12*BS-0.05). Significantly different using a Wilcoxon Signed Ranks Test (Z = -2.547, p = 0.011).

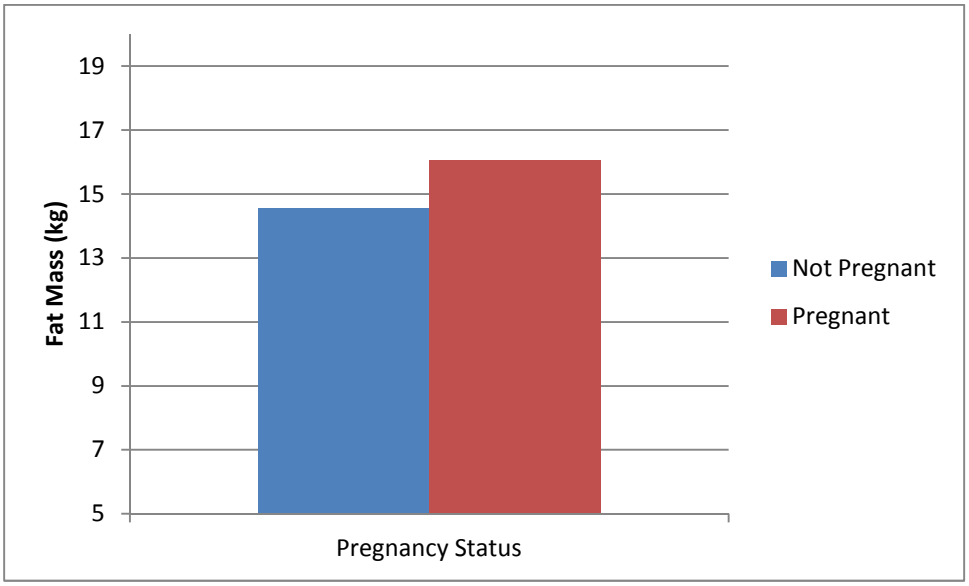

Figure 5.6. Mean fat mass (kg, triceps) in individuals by pregnancy status (n = 9). Not significantly different using a Wilcoxon Signed Ranks Test (Z = -1.481, p = 0.139).

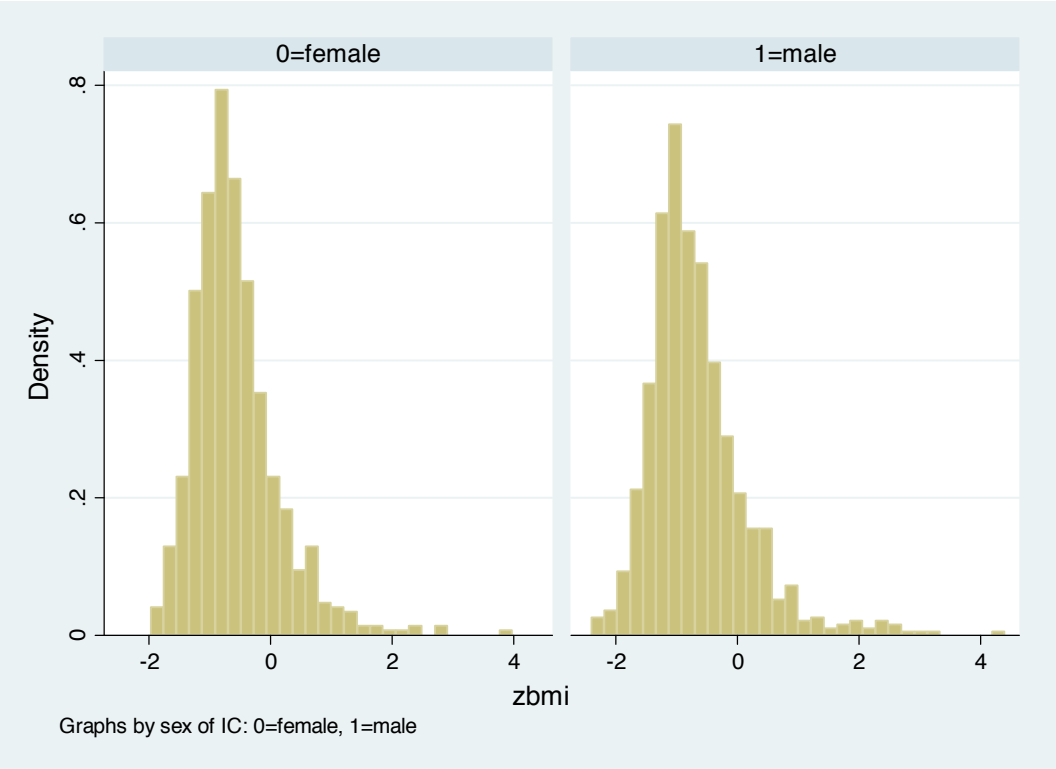

Figure 6.1a: Histogram of BMI Z-scores by sex (males n = 912, females n = 694).

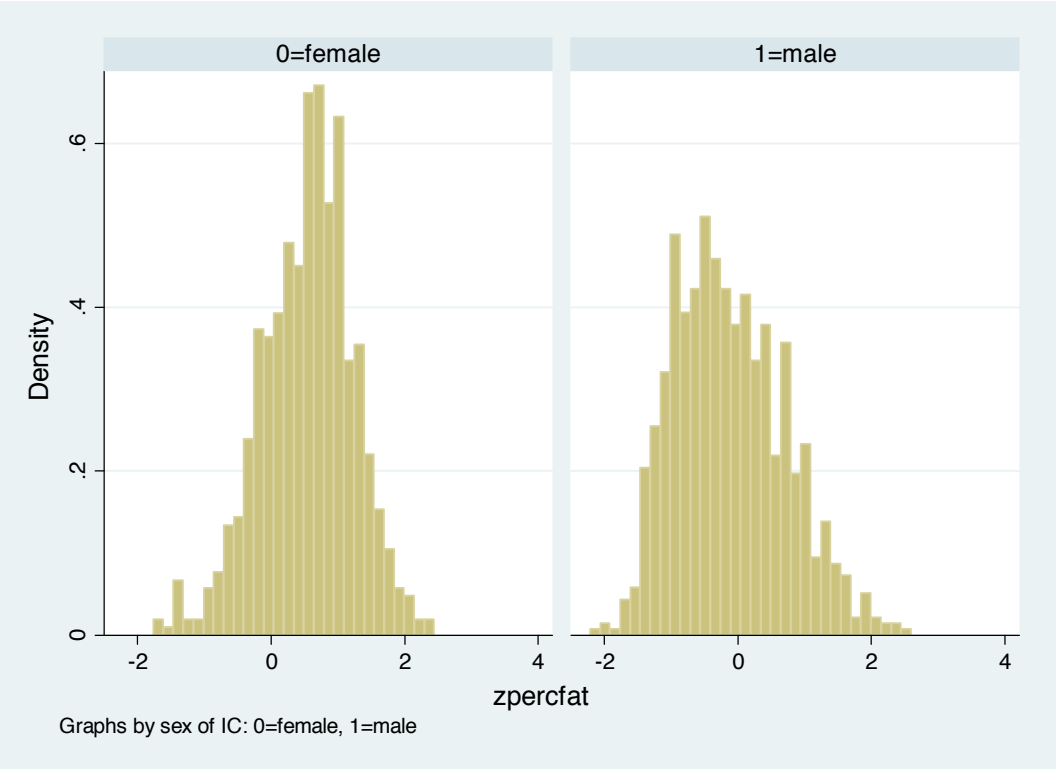

Figure 6.1b: Histogram of percent body fat z-scores by sex (males n = 912, females n = 694).

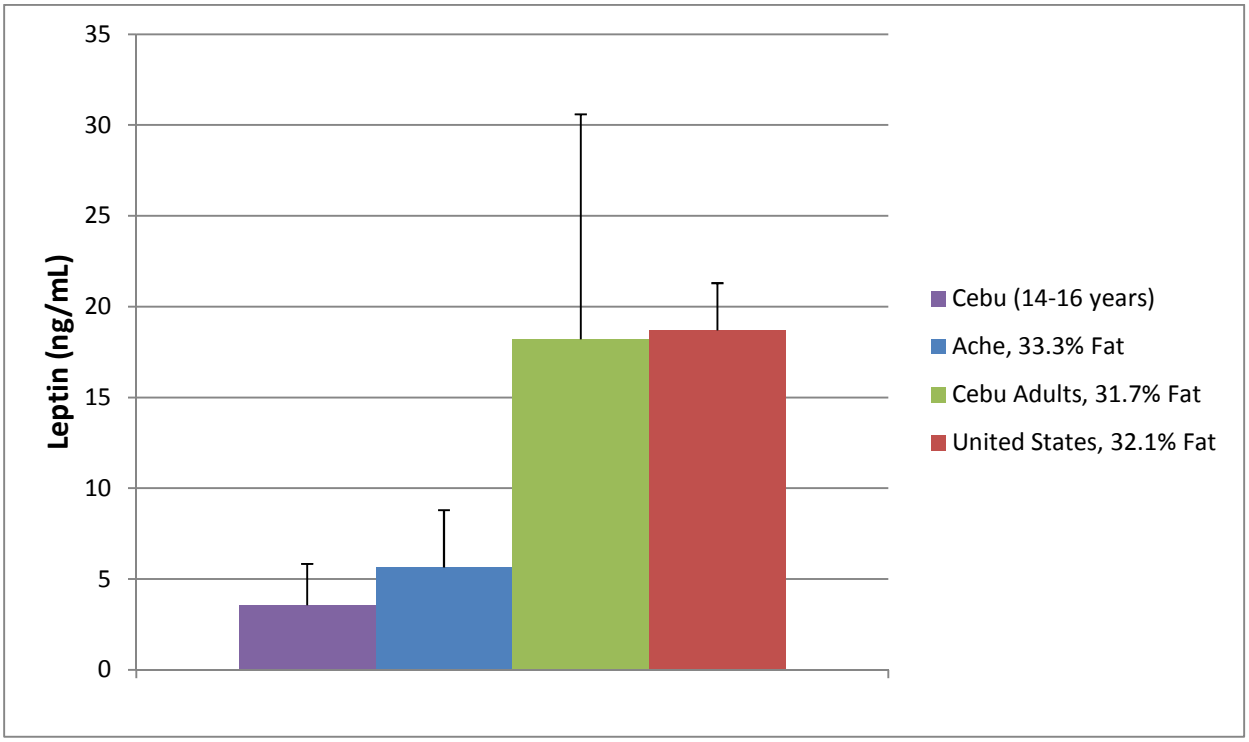

Figure 6.2a, Mean leptin levels for females in three different populations. Population labels include mean percent body fat values. Error bars on Cebu and Ache data represent standard deviation, on United states data they represent standard error. Cebu (14-16 years) data from Kuzawa et al. 2007, Ache data from Bribiescas 2001, United States data from Kennedy et al. 1997. Data on Cebu adolescent percent body fat not given.

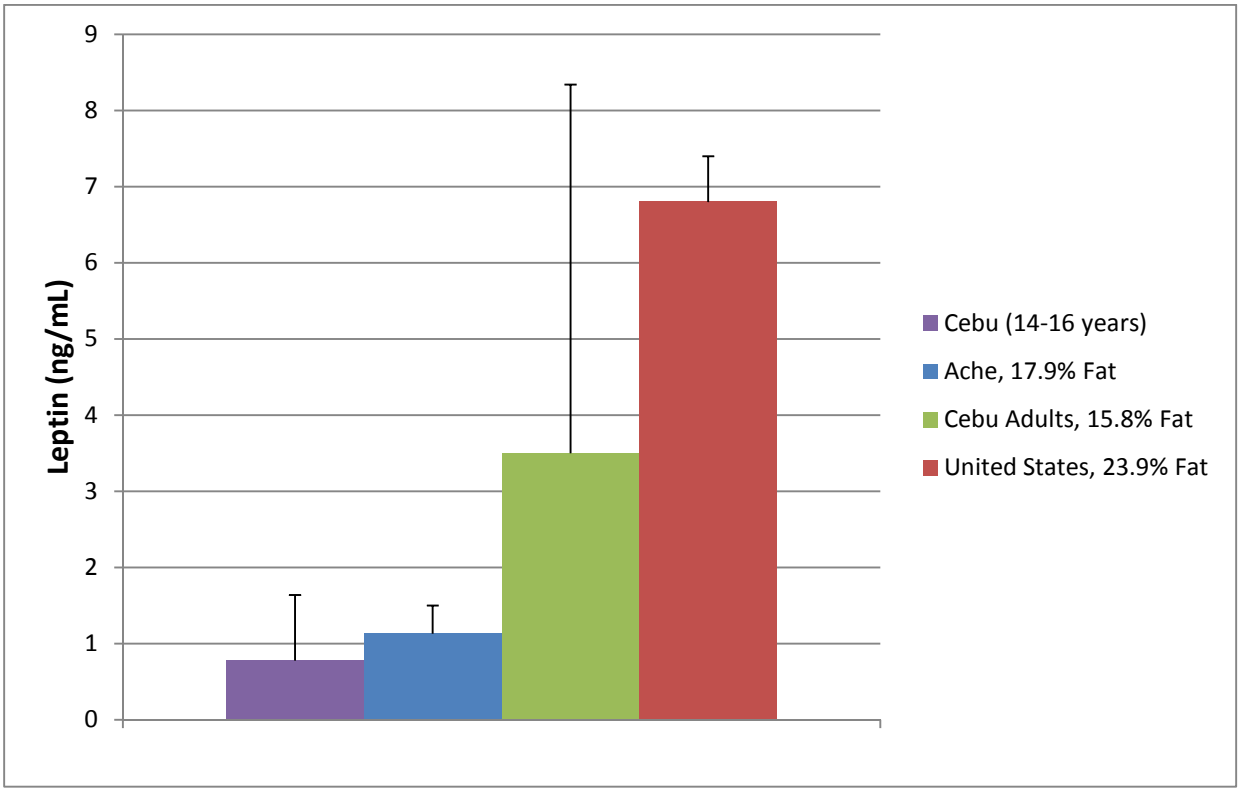

Figure 6.2b. Mean leptin levels for males in three different populations. Population labels include mean percent body fat values. Error bars on Cebu and Ache data represent standard deviation, on United states data they represent standard error. Cebu (14-16 years) data from Kuzawa et al. 2007, Ache data from Bribiescas 2001, United States data from Kennedy et al. 1997.

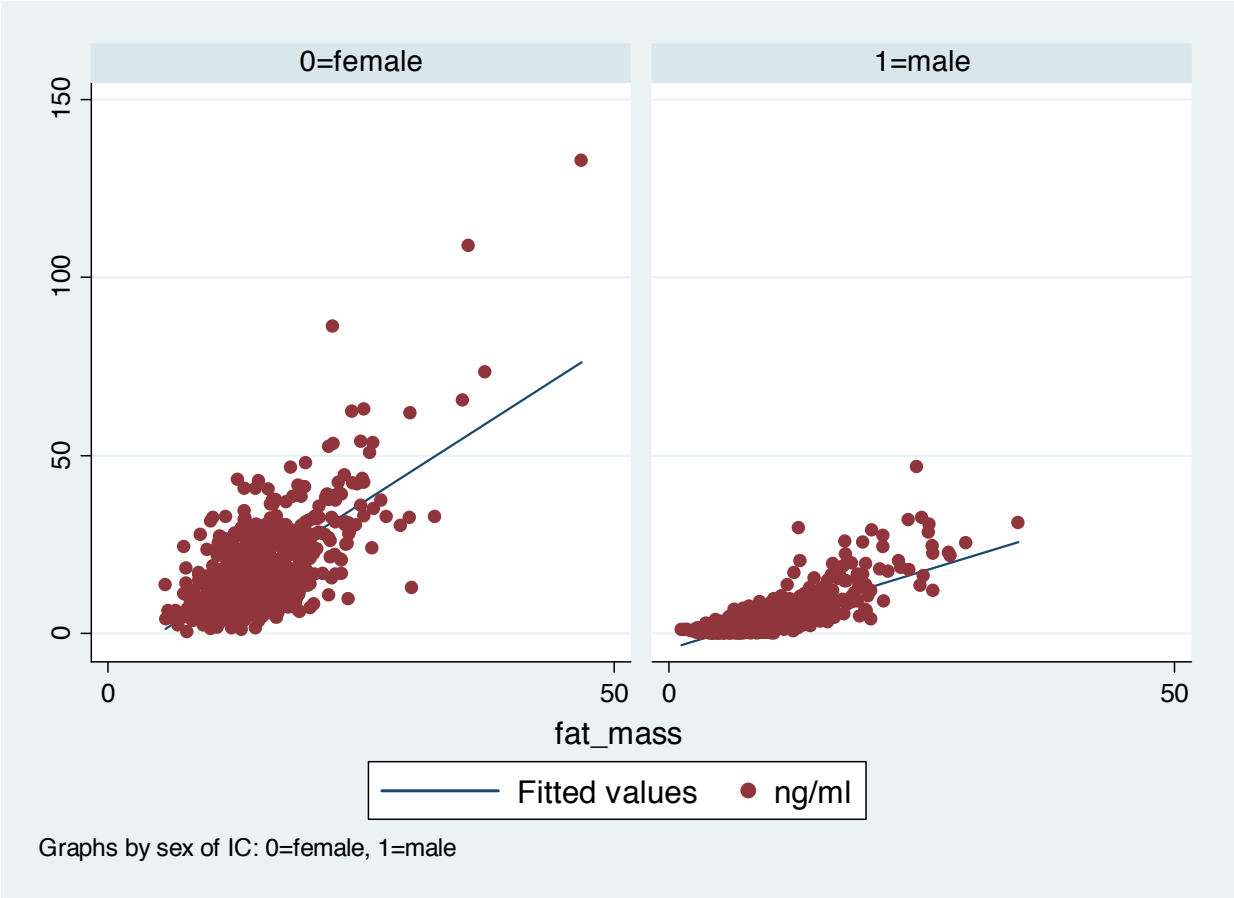

Figure 6.3. Relationship between leptin plasma levels (ng/mL) and fat mass (kg) by sex. For males (n = 912) leptin = 0.871*fat mass – 4.448. For females (n =694) leptin = 1.823*fat mass – 9.134.

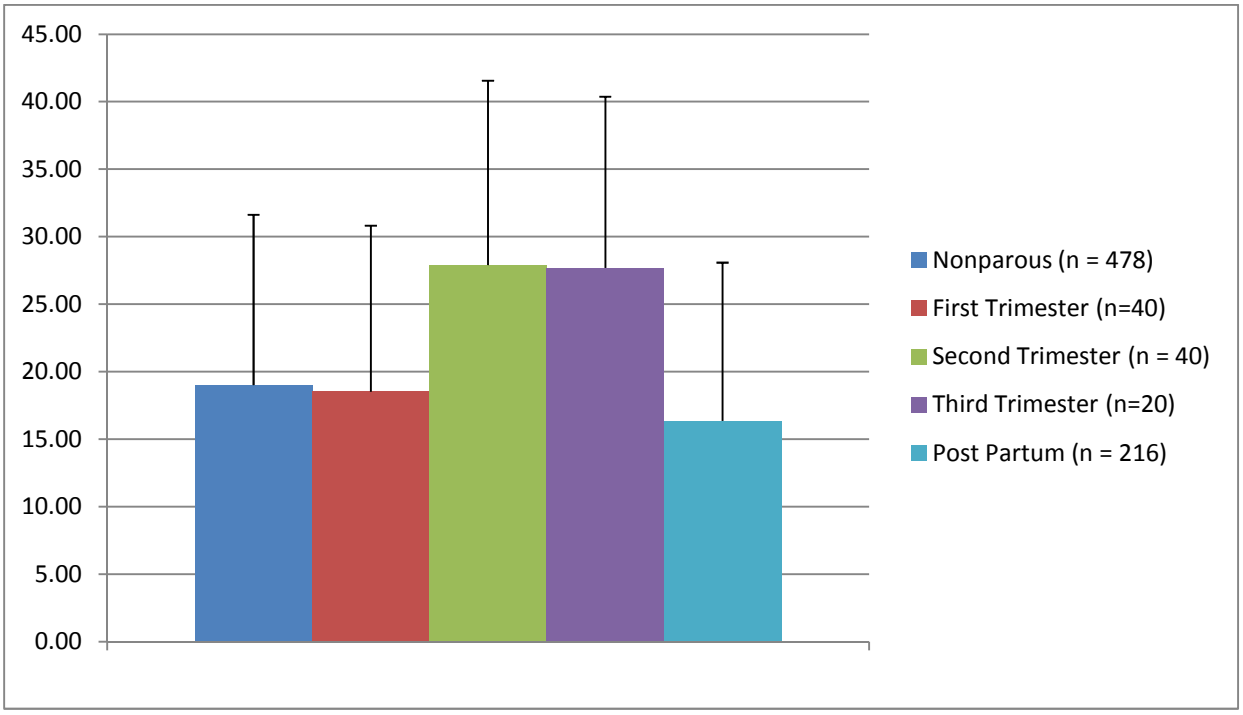

Figure 6.4. Mean plasma leptin levels across pregnancy states (n = 794). Kruskal-Wallis H test (H(4) = 46.616, p = 0.001).

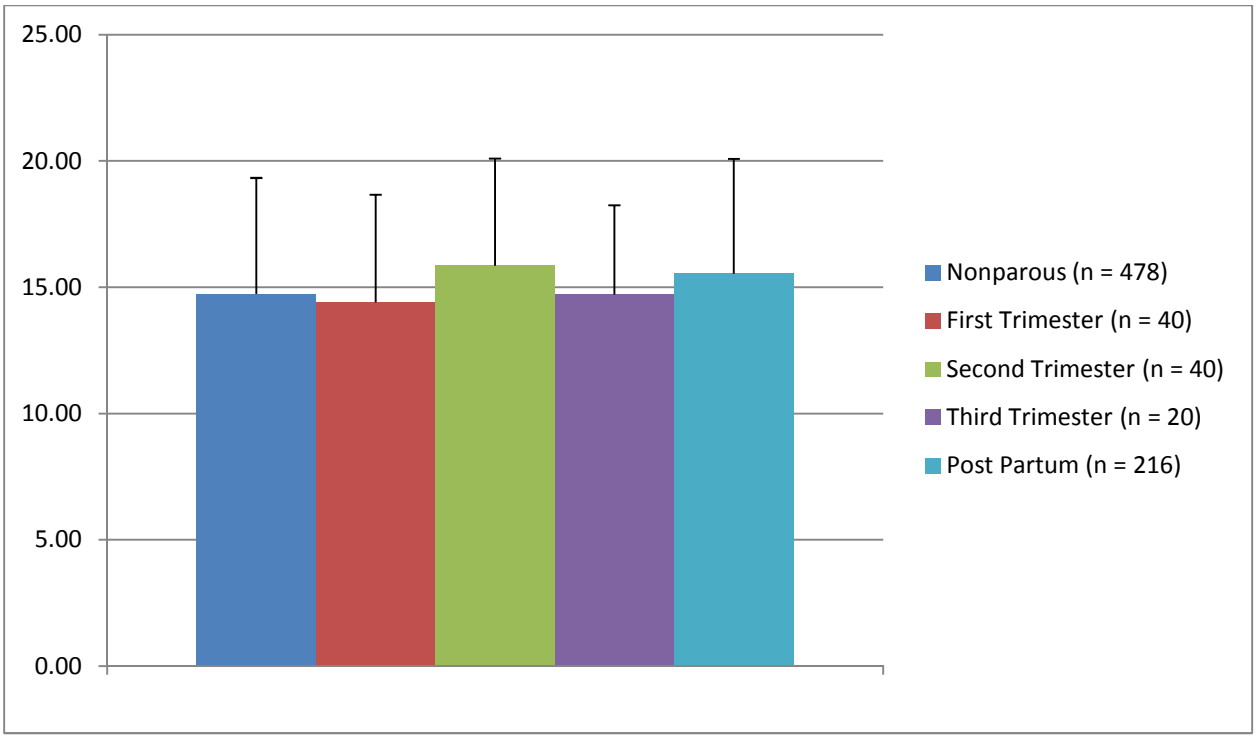

Figure 6.5. Mean fat mass (kg) across pregnancy states (n = 794). Kruskal-Wallis H test (H(4) = 9.812, p = 0.0437)

Tables

Table 1.1: Traits associated with leptin in genetically impaired Mice and Humans (synthesized from Himms-Hagen 1999).

Trait	*ob*/*ob* mice	Leptin deficient humans
Appetite	Hyperphagic	Hyperphagic
Adiposity	Increased	Increased
Female Sexual Maturation	None	None or long delay
Body Temperature	Lower	Normal
HPA Axis	Overactive	Normal
Cortisol	Overactive	Normal
HPT Axis	Marked Impairment	Mild Impairment
Growth	Stunted	Normal to mild impairment
Resting Energy Expenditure	Lower	Normal

Table 2.1a : Effects of weight loss on various biomarkers and the subsequent effect of leptin supplementation

Measure	Sex	Fasting/Weight loss	Leptin replacement	Reference
Leptin	M	decrease	restored	1
	F	decrease	restored	2, 3
Testosterone	M	decrease	restored	1
	F	no change	no effect	2
FSH	F	no change	no effect	2
Estradiol	F	no change	no effect	2
Estradiol	F	increased	no effect	3
LH pulsatility	M	decrease	normalized	1
Overnight LH Peak Frequency	F	decrease	normalized	2
LH pulse regularity (approx. entropy)	F	decrease	increased	3
SHBG	M	increase	no effect	1
T3, Triiodothyronine	M	decrease	no effect	1
	M/F	decrease	increase	4, 5
	F	decrease	no effect	2, 3
Reverse T3 (rT3)	M	increase	no effect	1
	F	increase	no effect	2, 3
T4, thyroxine	M	no change	small increase	1
	M/F	decrease	increase	4, 5
	F	no effect	no effect	2, 3
TSH	M	suppressed	blunted fall	1
	F	decreased	restored	3
	M/F	no change	no effect	4
GH	M, F	increase	no effect	1, 3
Total IGF-1	M	decrease	partialy normalized	1
Free IGF-1	M	decrease	no effect	1
IGF-1	F	decrease	no effect	2, 3
Cortisol (24 hour mean)	M, F	increase	no effect	1, 3
	F	no effect	no effect	2
Urinary epinephrine excretion	M	increase	decrease	1
	M/F	decreased	reversed	5

References: 1 = Chan et al. 2003, 2 = Chan et al. 2006, 3 = Schurgin et al. 2004, 4 = Rosenbaum et al. 2002, 5 = Rosenbaum et al. 2005

Table 2.1b: Effects of weight loss on various biomarkers and the subsequent effect of leptin supplementation

Measure	Sex	Fasting/Weight loss	Leptin replacement	Reference
Urinary norepinephrine exrcetion	M	non sig. increase	no effect	1
	M/F	decreased	no effect	5
Dopamine excretion	M/F	decreased	no effect	5
Total energy exp.	M/F	decrease	restores	4, 5
Resting metabolic rate (RMR)	M	increase	no effect	1
	F	no change	no effect	2, 3
	M/F	no change	no effect	4, 5
Thermic effect of feeding	M/F	no change	no effect	4, 5
Non-REE	M/F	decrease	restores	4, 5
Respiratory quotient (RQ)	M	decrease	no effect	1
	F	decrease	no effect	2
RQ at low leves of work	M/F	decrease	restores	5
RQ at high levels of work	M/F	no effect	no effect	5
Gross Mechanical efficiency (low work)	M/F	increase	restores	5
Gross Mechanical efficiency (high work)	M/F	no effect	no effect	5
24-hour urinary nitrogen	M	increase	no effect	1
Insulin	M	decrease	no effect	1
	F	decrease, non-sig decrease	no effect	2, 3
Sympathetic nervous system*	M/F	decreased activity	reversed	5
Parasympathetic nervous system**	M/F	increase activity	no effect	5
Free Fatty Acids	M	increased	no effect	1
	F	increased	no effect	2, 3
Hunger	F	increased	diminished	3
Appetite (visual analogue scale)	M	Increased	no effect	1

References: 1 = Chan et al. 2003, 2 = Chan et al. 2006, 3 = Schurgin et al. 2004, 4 = Rosenbaum et al. 2002, 5 = Rosenbaum et al. 2005

* measured by heart rate and catecholamine excretion

** measured by heart rate analysis

Table 3.2a: Leptin Levels in Comparative Populations, Males (Mean ±SD)

Num	Sample Description	n	Lifestyle	Age (yr)	BMI (kg/m^2)	Fat (%)	Fat Mass (kg)	Leptin (ng/mL)
1	Ache, Paraguay (Birbiescas 2001)	21	1	32.8 ± 15.7	23.8 ± 1.4	17.9 ± 1.8	10.1*	1.13 ± 0.37
2	Buryat, Siberia (Leonard et al. Unpublished)	41	1	29.8 ± 13.0	23.0 ± 3.9	17.8 ± 7.4	12.7 ± 8.1	2.4 ± 2.7
3	Danish, Caucasian (Echwald et al. 1999)	186	0	25.5 ± 3.4	24.1 ± 3.4	20 ± 6	16.3 ± 7.5	4.6 ± 3.1
4	Evenki, Siberia (Leonard et al. Unpublished)	25	1	38.3 ± 13.1	23.0 ± 2.8	14.1 ± 5.1	8.2 ± 4.2	1.25 ± 0.59
5	German (Neuhauser-Berthold et al. 2000)	82	0	69.0 ± 5.0	26.0 ± 2.6	31.8 ± 3.0	24.9 ± 4.5	4.7 ± 3.9
6	Indian, Asian (Banerji et al. 1999)	20	0	38.6 ± 10	24.5 ± 2.54	33.0 ± 7.0	23.2*	7.6 ± 3.3
7	Jamaican (Bennet et al. 1997)	144	0	N/A	24.2 ± 4.8	19.2 ± 8.0	14.6 ± 8.5	3.8 ± 3.6
8	Jamaican[1] (Luke et al. 1998)	144	0	46.3 ± 14.0	24.3 ± 4.8	18.5 ± 8.1	13.7 ± 8.4	3.9 ± 3.7
9	Mexican American, College (Peltz 2007)	110	0	22.1 ± 3.5	27.5 ± 5.3	42.6 ± 5.7	36.0 ± 10.9	17.4 ± 18.5
10	Nigerian[1] (Luke et al. 1998)	175	0	41.0 ± 19.5	20.8 ± 4.0	10.8 ± 8.0	7.2 ± 7.3	2.8 ± 2.8
11	Papua New Guinea , Huli (Tanaka et al. 2005)	20	1	30.0 ± 8.7	25.1 ± 2.3	18.8 ± 4.6	12.4*	3.1 ± 4.7
12	Papua New Guinea, Balopa(Tanaka et al. 2005)	20	1	30.5 ± 9.7	26.1 ± 4.4	19.6 ± 5.6	14.5*	3.5 ± 2.6
13	Phillipines, Cebu - 2005 Data, see chapter 6	912	0	21.5 ± 0.3	21.1 ± 3.1	15.8 ± 5.1	9.2 ± 4.4	3.6 ± 4.9
14	Poland, Students (Lutoslawaska et al. 2004)	17	0	22.5 ± 1.7	N/A	13.3 ± 3.4	10.4 ± 2.9	2.5 ± 1.0
15	Singapore (Ho et al. 1999)	69	0	41.3 ± 8.4	25.5 ± 3.3	24.3 ± 7.3	18.5*	9.1 ± 5.0
16	South Africa (Iputo et al. 2001)	56	1	26.8 ± 8.2	22.4 ± 3.9	13.9 ± 5.0	9.4 ± n/a	5.2 ± n/a
17	United States[2] (US) (Hickey et al. 1996)	333	0	39.1 ± 0.7	28.2 ± 0.3	22.3 ± 0.4	20.5 ± 0.5	6.9 ± 0.3
18	US[2] (Rosenbaum et al. 1996)	26	0	28 ± 1.3	28.0 ± 2.3	23.4 ± 2.6	24.7 ± 5.1	14.9 ± 5.0
19	US (Rosenbaum et al. 2001)	32	0	35.3 ± 13.5	24.4 ± 3.0	20.4*	15.7 ± 6.1	2.7 ± 1.7
20	US, African American (Considine et al. 2008)	37	0	33.9 ± 7.7	31. 0 ± 7.0	24.0 ± 7.3	24.7 ± 11.6	9.2 ± 7.2
21	US, African Origin[1] (Luke 1998)	275	0	39.3 ± 13.4	26.7 ± 5.0	25.0 ± 8.4	20.9 ± 10.9	6.8 ± 5.7
22	US, Caucasian (Hickey et al. 1997)	9	0	48.8 ± 1.8	N/A	28.4 ± 0.9	26.99 ± 2.37	9.88 ± 1.26
23	US, Caucasian (Perusse et al. 1997)	51	0	N/A	25.5 ± 5.0	21.0 ± 8.1	17.1 ± 11.1	4.6 ± 4.4
24	US, European American (Kalhan et al. 2001)	19	0	23.9 ± 2.3	24.1 ± 2.5	16.6*	13.3*	2.76 ± 1.29
25	US, South Asian American (Kalhan et al. 2001)	20	0	24.2 ± 1.9	22.0 ± 2.9	18.6*	12.4*	4.31 ± 2.47

Lifestyle: 1 = rural/subsistence, 0 = urban/market

[1](Self Identified of African origin)

[2](± SE used instead of SD)

*Value calculated

Table 3.2b: Leptin Levels in Comparative Populations, Females (Mean ± SD)

Num	Sample Description	N	Lifestyle	Age (yr)	BMI (kg/m^2)	Fat(%)	Fat Mass (kg)	Leptin (ng/mL)
1	Ache Amerindians, Paraguay (Birbiescas 2001)	12	1	32.2 ± 14.0	25.2 ± 1.9	33.3 ± 4.4	18.4*	5.64 ± 3.16
2	Buryat S. Siberia (Leonard et al. Unpublished)	53	1	29.4 ± 11.1	23.3 ± 4.5	34.8 ± 9.3	21.3 ± 9.5	7.7 ± 5.0
3	Canadian, Aboriginal (Silha et al. 2007)	131	0	41.1 ± 10.2	29.20 ± 5.87	37.8*	29.37 ± 9.74	33.77 ± 24.21
4	Canadian, Caucasian (Silha et al. 2007)	132	0	45.6 ± 14.1	27.52 ± 6.29	36.2*	26.78 ± 10.63	33.93 ± 25.90
5	Danish, Caucasian (Echwald et al. 1999)	194	0	25.0 ± 3.5	22.9 ± 3.8	26 ± 7	17.7 ± 7.9	15.0 ± 10.7
6	Ecuador, Shuar (Lindgarde et al. 2004)	26	1	32.0 ± 2.2	23.1 ± 2.8	22.1 ± 4.9	11.7 ± 3.3	4.1 ± 2.7
7	Ecuador, Yuwientsa (Lindgarde et al. 2004)	26	1	36.9 ± 2.1	24.1 ± 2.7	25.4 ± 4.9	14.5 ± 4.0	5.5 ± 3.1
8	Evenki Siberia (Leonard et al. Unpublished)	12	1	33.7 ± 13.1	24.4 ± 5.3	30.9 ± 7.0	17.0 ± 7.5	1.31 ± 0.28
9	German (Neuhauser-Berthold et al. 2000)	122	0	69.0 ± 6	26.3 ± 3.6	44.3 ± 3.4	29.9 ± 5.8	18.7 ± 12.3
10	Jamaican (Bennet et al. 1997)	228	0	N/A	28.6 ± 6.2	36.9 ± 8.6	28.4 ± 12.0	18.5 ± 13.9
11	Jamaican[1] (Luke et al. 1998)	226	0	47.0 ± 13.6	28.7 ± 6.3	36.3 ± 8.4	27.7 ± 11.8	18.6 ± 13.9
12	Japanese, Premenopausal (Douchi 2002)	75	0	41.2 ± 6.8	23.2 ± 4.1	33.6 ± 8.7	19.1 ± 7.2	8.4 ± 4.8
13	Japanese, Postmenopausal (Douchi 2002)	75	0	61.7 ± 7.1	24.1 ± 3.1	35.6 ± 7.5	19.6 ± 5.5	9.2 ± 7.1
14	Korean, Premenopausal (Kim et al. 2008)	145	0	40.8 ± 4.3	21.5 ± 2.5	27.6*	15.0 ± 4.0	6.3 ± 3.7
15	Korean, Postmenopausal (Kim et al. 2008)	118	0	57.7 ± 6.4	23.5 ± 2.9	31.4*	17.8 ± 4.6	6.7 ± 4.5
16	Mexican American, College (Peltz 2007)	242	0	22.1 ± 3.6	26.1 ± 6.3	49.1 ± 3.7	33.2 ± 9.7	60.8 ± 46.6
17	Nigeria[1] (Luke et al. 1998)	188	0	42.2 ± 17.7	22.8 ± 5.1	25.3 ± 8.4	14.4 ± 9.2	10.3 ± 8.3
18	Papua New Guinea, Huli (Tanaka et al. 2005)	17	1	34.1 ± 7.5	29.7 ± 4.7	33.3 ± 5.0	22.6*	19.7 ± 11.9
19	Papua New Guinea, Balopa (Tanaka et al. 2005)	17	1	33.7 ± 8.9	26.4 ± 4.9	34.1 ± 6.2	22.0*	22.7 ± 12.9
20	Peruvian, Cuzco (Lindgarde et al. 2004b)	105	1	35 ± 10	24.6 ± 3.9	31.2 ± 4.8	17.7 ± 5.3	9.7 ± 6.5
21	Peruvian, Lima (Lindgarde et al. 2004b)	105	1	37 ± 10	25.1 ± 4.7	34.1 ± 4.8	21.5 ± 5.8	14.0 ± 0.7
22	Phillipines, Cebu - 2005 Data, see chapter 6	694	0	21.5 ± 0.3	20.3 ± 3.2	31.7 ± 4.8	15.0 ± 4.6	18.2 ± 12.4
23	Poland, Students (Lutoslawaska et al. 2004)	19	0	22.1 ± 0.8	N/A	20.0 ± 4.3	11.9 ± 3.6	6.9 ± 2.5
24	Singapore (Ho et al. 1999)	64	0	40 ± 7.8	25.5 ± 4.6	35.4 ± 5.8	22.8*	21.6 ± 10.8
25	South Africa (Iputo et al. 2001)	79	1	30.9 ± 14.7	26.1 ± 6.3	26.3 ± 9.1	16.9 ± n/a	13.5 ± n/a
26	United States (US) (Ryan et al. 2003)	148	0	50 ± 1	28.1 ± 0.5	37.2 ± 1.1	28.0*	20.0 ± 1.3
27	US[2] (Hickey et al. 1996)	63	0	34.4 ± 1.2	25.6 ± 0.6	28.1 ± 1.2	20.4 ± 1.5	15.2 ± 1.3
28	US, African American (Considine et al. 2008)	32	0	32.2 ± 7.4	28.9 ± 7.1	34.1 ± 7.9	28.7 ± 14.2	23.2 ± 15.8
29	US, African origin[1] (Luke 1998)	275	0	43.1 ± 15.1	30.7 ± 7.9	40.4 ± 8.7	33.8 ± 15.2	27.7 ± 19.5

Table 3.2b continued

Num	Sample Description	N	Lifestyle	Age(yr)	BMI (kg/m^2)	Fat(%)	Fat Mass (kg)	Leptin (ng/mL)
30	US, Caucasian (Hickey et al. 1997)	9	0	45.6 ± 1.7	N/A	30.5 ± 1.8	21.83 ± 2.25	18.27 ± 2.55
31	US, Caucasian (Perusse et al. 1997)	51	0	N/A	23.0 ± 3.5	25.5 ± 7.8	16.6 ± 7.6	11.9 ± 8.5
32	US, European American (Kalhan et al. 2001)	29	0	24.0 ± 2.4	22.9 ± 5.1	29.1*	17.8*	10.24 ± 6.25
33	US, Premenopausal (Blum et al. 2003)	153	0	41.6 ± 0.8	24.8 ± 5.2	33 ± 9	21.7*	14.6 ± 10.2
34	US, Premenopausal (Rosenbaum et al. 2001)	26	0	30.2 ± 8.8	22.5 ± 2.3	28.8*	17.0 ± 4.6	9.1 ± 5.7
35	US[2], Premenopausal (Rosenbaum et al. 1996)	20	0	27 ± 2.0	30.5 ± 2.6	38.1 ± 2.9	35.2 ± 6.0	46.3 ± 2.1
36	US[2], Postmenopausal (Rosenbaum et al. 1996)	21	0	66 ± 2.0	26.0 ± 0.9	37.4 ± 1.4	25.0 ± 1.6	41.2 ± 1.6
37	US, South Asian American (Kalhan et al. 2001)	32	0	23.2 ± 2.2	22.3 ± 3.4	29.4*	16.4*	20.56 ± 10.3

Lifestyle: 1 = rural/subsistence, 0 = urban/market

[1](Self Identified of African origin)

[2](± SE used instead of SD)

*Value Calculated

Table 3.3: Regression models of various anthropometric measures with log leptin

Regression Model	Total (n = 62)	ß, standardized (95%)	P-value	R^2
1	Fat Mass	0.64 (0.50, 0.78)	<0.000	0.75
	Sex	0.40 (0.26, 0.54)	<0.000	
2	Fat percentage	0.68 (0.47, 0.89)	<0.000	0.64
	Sex	0.17 (-0.04, 0.38)	0.114	
	Males (n = 25)			
3	Fat mass	0.83 (0.59, 1.07)	<0.000	0.69
4	Fat percentage	0.71 (0.41, 1.01)	<0.000	0.51
7	Fat mass	0.74 (0.48, 1.00)	<0.000	0.72
	Lifestyle	-0.20 (-0.46, 0.07)	0.134	
	Females (n = 37)			
5	Fat mass	0.74 (0.51, 0.97)	<0.000	0.54
6	Fat percentage	0.61 (0.34, 0.88)	<0.000	0.37
8	Fat mass	0.66 (0.43, 0.89)	<0.000	0.60
	Lifestyle	-0.25 (-0.48, -0.01)	0.038	

Table 5.1: Schedule of research activities

Time Schedule	Research Activities
August 22 to December 1st 2004	Arrival Bolivia + Project set-up: Tsimane council permission, preliminary community meetings, Initial training of translators/research assistants
December 1st - February 2005	Round I: Wet Season
May 28th to August 29th 2005	Round II: Dry Season, addition of Alta Gracia community, leave Bolivia
January 10 to March 18th 2006	Round III: Wet Season (return to Bolivia)
July/August 2006	Leptin Blood Spot Analysis (LHBR at Northwestern)

Table 5.2. Descriptive statistics for Tsimane' adults, presented as means (SD).

Measure	Females (n = 36)	Males (n=30)
Age (years)	31.1 (11.5)	35.6 (12.5)
Height (cm)	153.4 (3.4)**	164.2 (5.0)
Weight (kg)	54.0 (7.0)**	63.4 (6.5)
Body Mass Index (kg/m^2)[1]	22.9 (2.7)	23.5 (1.7)
Percent Overweight	19.4	23.3
Percent Obese	2.8	0
Leptin (BS, ng/mL)[1]	1.183 (0.878)**	0.145 (0.170)
Leptin (PL, ng/mL)[1]	2.458 (1.859)**	0.261(0.361)
Body Fat (SOS; %)	30.9 (4.6)**	17.8 (4.5)
Waist circumference (cm)[1]	80.21 (7.44)	81.88 (4.97)
Hip circumference (cm)[1]	91.22 (5.67)	91.51 (4.21)
Midarm circumference (cm)[1]	26.69 (3.05)*	27.51 (1.80)
Triceps skinfold (mm)[1]	17.09 (4.66)**	8.52 (2.97)
Biceps skinfold (mm)[1]	8.15 (3.29)**	4.58 (1.81)
Subscapular skinfold (mm)[1]	18.04 (5.50)**	12.00 (2.97)
Suprailiac skinfold (mm)[1]	19.14 (6.21)**	13.17 (5.48)
Sum of 4 Skinfolds	62.42 (16.76)**	38.27 (10.97)
Body Fat (BIA, %)	24.87 (6.29)**	17.13 (3.92)
Body Fat (TriSF, %)	29.03 (4.11)**	17.74 (4.13)
Body Fat (SOS, %)	30.89 (4.62)**	17.80 (4.52)
Fat Mass (BIA, kg)	13.80 (5.21)	10.99 (3.21)
Fat Mass (Triceps, kg)[1]	15.89 (4.19)**	11.33 (3.20)
Fat Mass (SOS, kg)[1]	16.91 (4.62)**	11.40 (3.57)
Waist-Hip Ratio	0.88 (0.05)	0.90 (0.05)
Arm Muscle Area[1]	30.06 (8.05)**	39.34 (7.08)

Differences between male and females are statistically significant at *P<0.05, **P<0.001

[1]Uses Mann-Whitney U instead of Independent T-Test because of non-normal data

Table 5.3. Nutritional indices in Tsimane'.

	Females (n=36)		Males (n=30)	
Measurement	Mean (SD)	% < 2 SD[2]	Mean (SD)	% < 2 SD
Height for Age Z-Score	-1.44 (0.54)	11.11*	-1.68 (0.77)	43.33
Weight for Age Z-Score	-0.67 (0.48)*	0.00	-1.07 (0.50)	3.33
Weight for Height Z-Score	-0.58 (0.48)	0.00	-0.53 (0.41)	0.00
Arm Muscle Area Z-Score[1]	-0.18 (0.84)**	0.00	-1.25 (0.63)	10.00

Differences between male and females are statistically significant at: *P < 0.01, **P < 0.001
[1]Uses Mann-Whitney U instead of Independent T-Test because of non-normal data.
[2] Chi-square used

Table 5.4. Pearson correlations between leptin (ng/mL, logged) and measures of adiposity

	Female (n=36)		Male (n=30)	
	r	p-value	r	p-value
Triceps Skinfold*	0.553	0.000	0.553	0.002
Biceps Skinfold*	0.376	0.024	0.016	0.932
Subscapula Skinfold*	0.294	0.082	0.578	0.001
Suprailiac Skinfold*	0.460	0.005	**0.655**	0.000
Sum of 4 Skinfold	0.515	0.001	0.622	0.000
Body fat % (BIA)	0.510	0.001	0.360	0.051
Fat mass (BIA)	0.559	0.000	0.384	0.036
Body fat % (Triceps sf)	0.556	0.000	0.555	0.001
Fat mass (Triceps sf)*	**0.575**	0.000	0.555	0.001
Body fat % (Sum of 4)	0.436	0.008	0.527	0.003
Fat mass (sum of 4)*	0.520	0.001	0.553	0.002
Body Mass Index*	0.550	0.001	0.301	0.107

*Variables were logged transformed to normalize data.

Table 5.5. Regression models, Leptin (logged) as dependant variable.

Model	N	Total	ß (SE)	P-value	r^2	Adjusted r^2
1	66	Fat Mass (Triceps)*	1.880 (0.354)	<0.000	0.746	0.738
		Sex	-.750 (0.098)	<0.000		
		Constant	-2.263 (0.424)	<0.000		
2	66	Body fat % (Triceps)	0.052 (0.010)	<0.000	0.740	0.732
		Sex	-0.442 (0.143)	<0.000		
		Constant	-1.553 (0.303)	0.003		
3	42	Fat Mass (Triceps)*	1.529 (0.430)	0.001	0.240	0.221
		Constant	-1.780 (0.506)	0.001		
4	42	Fat Mass (Triceps)*	1.530 (0.368)	<0.000	0.457	0.429
		Pregnant	0.342 (0.087)	<0.000		
		Constant	-1.862 (0.434)	<0.000		

*Logged

Table 6.1. Sample characteristics

	Mean and Standard Deviations[a, b]					
Variable	Male (n = 912)		Female (n = 694)		Pregnant (n=100)	
Leptin (ng/ml)[c]	3.55	4.92	18.20	12.40	24.12	13.60
Age (years)	21.48	0.30	21.48	0.32	21.43	0.28
Height (cm)	163.02	5.89	151.26	5.48	150.83	5.31
Weight (kg)	56.07	9.37	46.47	8.20	47.06	7.62
BMI (kg/m^2)	21.06	3.05	20.29	3.23	20.66	3.06
Overweight (%)[d,e]	7.24	66	6.34	44	7.00	7
Obesity (%)[d,e]	2.08	19	1.44	10	1.00	1
Underweight (%)[d,e]	15.46	141	28.82	200	27.00	27
Waist/hip ratio	0.95	0.04	0.88	0.04	N/A	N/A
Triceps Skinfold[c]	10.89	5.49	20.81	6.35	19.97	5.67
Subscapular Skinfold[c]	12.46	5.55	18.80	6.66	18.65	6.06
Surpailiac Skinfold[c]	14.38	8.09	22.52	8.12	22.45	8.04
Bodyfat (%)[c]	15.80	5.10	31.73	4.81	31.53	4.64
Fat Mass (kg)[c]	9.18	4.43	14.99	4.59	15.05	4.14

[a]All sex differences significant at $p < 0.000$ except age, percent overweight, and percent obese.
[b]In females pregnancy status differences significant at $p < 0.001$ for leptin, all other variables not significant.
[c]Values log transformed for t-tests to determine sex and pregnancy status differences .
[d]Chi-square used to test for sex and pregnancy status differences, Fisher's exact test used for frequencies less than 5.
[e]Counts (n) provided instead of SD.

Table 6.2. Nutritional indices in Filipino Adults

	Males (n=912)		Females (n=694)	
Measurement	Mean (SD)	% < 2 SD[1]	Mean (SD)	% < 2 SD
Height for Age Z-Score	-1.94 (0.84)**	46.82*	-1.81 (0.84)	40.49
Weight for Age Z-Score	-1.33 (0.70)***	12.94***	-1.12 (0.64)	3.60
Weight for Height Z-Score	-1.11 (0.73)	5.04**	-1.09 (0.58)	2.16
Arm Muscle Area Z-Score	-1.33 (0.68)***	15.68***	-0.92 (0.85)	4.76
BMI Z-Score	-0.67 (0.80)**	1.21**	-0.57 (0.70)	0.00
Percent Fat Z-Score	-0.11 (0.82)***	0.33	0.54 (0.69)	0.00
Triceps skinfold Z-score	-0.06 (0.86)***	0.00	0.10 (0.77)	0.00

Differences between male and females are statistically significant at: *P < 0.05, ** p < 0.01, ***P < 0.001

[1] Chi-square used, Fisher's exact test used for low BMI z-score and % BF z-score

Table 6.3 Pearson correlation coefficients (r) relating various measures of body composition with leptin[a] (ng/ml)

	Males (n = 912)	Females (n = 694)	Pregnant Females (n = 100)
Fat mass (kg)[a]	0.62***	0.59***	0.47***
BMI (kg/m^2)	0.52***	0.52***	0.45***
Body fat (%)[a]	0.60***	0.54***	0.37***
Waist/hip ratio	0.07*	0.00	N/A
Triceps skinfold (mm)[a]	0.60***	0.53***	0.35***
Subscapula skinfold (mm)[a]	0.59***	0.51***	0.34***
Suprailiac skinfold (mm)[a]	0.59***	0.48***	0.37***
Height (cm)	0.12***	0.10*	0.160
Weight (kg)	0.51***	0.51***	0.47***
Waist circumference (cm)	0.56***	0.52***	N/A
Hip circumference (cm)	0.56***	0.57***	N/A

[a]log transformed

*p < 0.05, **p < 0.01, ***p < 0.001

Table 6.4 Regression slope coefficients relating various measures of body composition with leptin[a] (ng/ml)

	Males (n = 912)	Females (n = 694)	P-Value for interaction[b]
	B (SE)	B (SE)	
Fatmass (kg)[a]	1.90 (0.08)***	1.39 (0.07)***	0.000
BMI (kg/m^2)	0.24 (0.01)***	0.11 (0.01)***	0.000
Body fat (%)[a]	2.45 (0.11)***	2.33 (0.13)***	0.599
Waist/hip ratio	2.24 (1.09)*	0.08 (0.69)	0.141
Triceps (mm)[a]	1.84 (0.08)***	1.16 (0.07)***	0.000
Subscapula (mm)[a]	2.21 (0.10)***	1.00 (0.06)***	0.000
Suprailiac (mm)[a]	1.51 (0.07)***	0.82 (0.06)***	0.000
Height (cm)	0.03 (0.01)***	0.01 (0.00)**	0.100
Weight (kg)	0.08 (0.00)***	0.04 (0.00)***	0.000
Waist circumference (cm)	0.10 (0.01)***	0.05 (0.00)***	0.000
Hip circumference (cm)	0.11 (0.01)***	0.05 (0.00)***	0.000

[a]log transformed

[b]Presents the p-value for an interaction between the body composition variable and sex

*p < 0.05, **p < 0.01, ***p < 0.001

REFERENCES

Adair LS, Popkin BM, Akin JS, Guilkey DK, Gultiano S, Borja J, Perez L, Kuzawa CW, McDade T, Hindin MJ. 2011. Cohort Profile: The Cebu Longitudinal Health and Nutrition Survey. International Journal of Epidemiology. 40 (3): 619-625.

Adair LS and Popkin BM. 2005. Are Child Eating Patterns Being Transformed Globally. Obesity Research. 13 (7): 1281-1299.

Adair LS. 2004. Dramatic rise in overweight and obesity in adult filipino women and risk of hypertension. Obesity research 12(8):1335-1341.

Adair LS and Guilkey DK. 1997. Age-Specific Determinants of Stunting in Filipino Children. Journal of Nutrition. 314-320.

Ahima RS, Flier JS. 2000. Adipose tissue as and endocrine organ. Trends in endocrinology and metabolism. 327-32.

Ahima RS. 2008. Revisiting leptin's role in obesity and weight loss. The Journal of clinical investigation 118(7):2380-2383.

Ahima RS, Prabakaran D, Mantzoros C, et al. 1996. Role of leptin in the neuroendocrine response to fasting. Nature. 382 (6588): 250-2.

Alexe D-M and Petridou E. 2006. Leptin and Cancer. In: Leptin. Castracane VD and Henson MC eds. Singer, New York. 201-223.

Alexander C, Cochran CJ, Gallicchio L, Miller SR, Flaws JA, and Zacur H. 2010. Serum leptin levels, hormone levels, and hot flashes in midlife women. Fertility and sterility 94(3):1037-1043.

Al-Harithy RN. 2004. Relationship of leptin concentration to gender, body mass index and age in Saudi adults. Saudi medical journal 25(8):1086-1090.

Anim-Nyame N, Sooranna SR, Steer PJ, Johnson MR. 2000. Longitudinal analysis of maternal plasma leptin concentrations during normal pregnancy and pre-eclampsia. Human Reproduction. 15(9): 2033-2036.

Arabi A. 2005. Leptin effect on bone: is it ethnic specific? The Journal of laboratory and clinical medicine 146(1):7-8.

Arch JR, Stock MJ, Trayhurn P. 1998. Leptin resistance in obese humans: does it exist and what does it mean? In J Obes Relat Metab Disord. 22(12): 1159-63.

Ategbo JM, Grissa O, Yessoufou A, et al. 2006. Modulation of adipokines and cytokines in gestational diabetes and macrosomia. J Clin Endocrinol Metab. 91(10): 4137-4143.

Augustine RA, Ladyman SR, and Grattan DR. 2008. From feeding one to feeding many: hormone-induced changes in bodyweight homeostasis during pregnancy. The Journal of physiology 586(2):387-397.

Aydin S, Guzel SP, Kumru S, et al. 2008. Serum leptin and ghrelin concentrations of maternal serum, arterial and venous cord blood. Journal of physiology and biochemistry. 64 (1): 51-59.

Bado S, Kermorgant S, Laigneau JP, Bortoluzzi MN, Moizo L, Lehy T, Guerre-Millo M, Le Marchand-Brustel Y and others. 1998. The stomach is a source of leptin. Nature 394(6695):790-793.

Baksu A, Ozkan A, Goker N, et al. 2005. Serum leptin levels in the Preeclamptic pregnant women: relationship to thyroid-stimulating hormone, body mass index, and proteinuria. American Journal of Perinatology. 22(3): 161-164.

Balligand JL, Brichard SM, Brichard V, Desager JP, and Lambert M. 1998. Hypoleptinemia in patients with anorexia nervosa: loss of circadian rhythm and unresponsiveness to short-term refeeding. European journal of endocrinology / European Federation of Endocrine Societies 138(4):415-420.

Banerji MA, Faridi N, Atluri R, Chaiken RL, and Lebovitz HE. 1999. Body composition, visceral fat, leptin, and insulin resistance in Asian Indian men. The Journal of clinical endocrinology and metabolism 84(1):137-144.

Banks WA, Phillips-Conroy JE, Jolly CJ, and Morley JE. 2001. Serum leptin levels in wild and captive populations of baboons (papio): implications for the ancestral role of leptin. The Journal of clinical endocrinology and metabolism 86(9):4315-4320.

Bates SH and Myers MG. 2003. The role of leptin receptor signaling in feeding and neuroendocrine function. Tends Endocrinol Metab. 14(10): 447-52.

Bell LN and Considine RV. 2006. Leptin and Obesity. In: Leptin. Castracane VD and Henson MC eds. Singer, New York. 33-51.

Bennett FI, McFarlane-Anderson N, Wilks R, Luke A, Cooper RS, and Forrester TE. 1997. Leptin concentration in women is influenced by regional distribution of adipose tissue. The American journal of clinical nutrition 66(6):1340-1344.

Bergendahl M, Evans WS, Pastor C, Patel A, Iranmanesh A, and Veldhuis JD. 1999. Short-term fasting suppresses leptin and (conversely) activates disorderly growth hormone secretion in midluteal phase women--a clinical research center study. The Journal of clinical endocrinology and metabolism 84(3):883-894.

Bernotiene E, Palmer G, and Gabay C. 2006. The role of leptin in innate and adaptive immune responses. Arthritis Res Ther. 8(5).

Berti PR, and Leonard WR. 1998. Demographic and socioeconomic determinants of variation in food and nutrient intake in an Andean community. American journal of physical anthropology 105(4):407-417.

Bjorbaek C, Buchholz RM, Davis SM, et al. 2000. Divergent roles of SHP-2 in ERK activation by leptin receptors. J Biol Chem. 276(7): 4747-55.

Black FL. 1977. Nutritional status of Braziliam Kayabpo Indians. Human Biology. 49(2):139.

Blain H, Vuillemin A, Guillemin F, Durant R, Hanesse B, De Talance N, Doucet B, and Jeandel C. 2002. Serum leptin level is a predictor of bone mineral density in postmenopausal women. J Clin Endocr Metab 87(3):1030-1035.

Blow AJ and Hartnett K. 2005. Infidelity in committed relationships I: A methodological review. Journal of Marital and Family Therapy. 31(2):183–216.

Blum M, Harris SS, Must A, Naumova EN, Phillips SM, Rand WM, and Dawson-Hughes B. 2003. Leptin, body composition and bone mineral density in premenopausal women. Calcified tissue international 73(1):27-32.

Boas F. 1911. Changes in the Bodily Form of Descendants of Immigrants. Senate Document 2008. Washington, DC. Government Printing Office, 61st Congress.

Boden G, Chen X, Mozzoli M, and Ryan I. 1996. Effect of fasting on serum leptin in normal human subjects. The Journal of clinical endocrinology and metabolism 81(9):3419-3423.

Boden G, Chen X, Kolaczynski JW, and Polansky M. 1997. Effects of prolonged hyperinsulinemia on serum leptin in normal human subjects. The Journal of clinical investigation 100(5):1107-1113.

Boesch C. 2010. Away from ethnocentrism and anthropocentrism: Towards a scientific understanding of "what makes us human"' Behavioral and Brain Sciences. 33 (2/3): 26-27.

Bornstein SR, Licinio J, Tauchnitz R, Engelmann L, Negrao AB, Gold P, and Chrousos GP. 1998. Plasma leptin levels are increased in survivors of acute sepsis: associated loss of diurnal rhythm, in cortisol and leptin secretion. The Journal of clinical endocrinology and metabolism 83(1):280-283.

Bouchard C. 2007. The biological predisposition to obesity: beyond the thrifty genotype scenario. Int J Obes (Lond) 31(9):1337-1339.

Bracken MB. 2009. Why are so Many Epidemiology Associations Inflated or Wrong? Does poorly conducted animal research suggest implausible hypotheses? Annals of Epidemiology. 19: 220-224.

Bray GA and Gallagher TF jr. 1975. Manifestations of hypothalamic obesity in man: a comprehensive investigation of eight patients and a review of the literature. Medicine. 54 (4): 301-30.

Briana DD and Malamitsi-Puchner A. 2009. Adipocytokines in Normal and Complicated Pregnancies. Reproductive Sciences. 1-17.

Bribiescas RG. 2001. Serum leptin levels and anthropometric correlates in Ache Amerindians of eastern Paraguay. American journal of physical anthropology 115(4):297-303.

Butler AA and Kozak LP. 2010. A Recurring Problem With the Analysis of Energy Expenditure in Genetic Models Expressing Leans and Obese Phenotypes. Diabetes. 59: 323-329.

Butte NF, Hopkins JM, Nicolson MA. 1997. Leptin in human reproduction: Serum Leptin Levels in Pregnant and Lactating Women. Journal of Clinical Endocrinology and Metabolism. 82(2): 585-589.

Brons C, Jensen CB, Storgaard H, Hiscock NJ, White A, Appel JS, Jacobsen S, Nilsson E, Larsen CM, Astrup A et al. . 2009. Impact of short-term high-fat feeding on glucose and insulin metabolism in young healthy men. The Journal of physiology 587(P10):2387-2397.

Byravan S. 1996. Can We Pop a Pill to Cure Obesity. Resonance. 69-72.

Byron E. 2003. Market Integration and Health: the Impact of Market and Acculturation on the Self-Perceived Morbidity, Diet, and Nutritional Status of the Tsimane' Amerindians of Lowland Bolivia PhD Dissertation, University of Florida.

Calissendorff J, Brismar K, and Rojdmark S. 2004. Is decreased leptin secretion after alcohol ingestion catecholamine-mediated? Alcohol Alcohol 39(4):281-286.

Campbell KL. 1994. Blood, Urine, Saliva and Dip-Sticks: Experiences in Africa, New Guinea, and Boston. Annals of the New York Academy of Sciences. 709: 312-329.

Carlson O, Martin B, Stote KS, Golden E, Maudsley S, Najjar SS, Ferrucci L, Ingram DK, Longo DL, Rumpler WV et al. 2007. Impact of reduced meal frequency without caloric restriction on glucose regulation in healthy, normal-weight middle- aged men and women. Metabolism: clinical and experimental 56(12):1729-1734.

Carlton ED, Demas GE, and French SS. 2012. Leptin, a neuroendocrine mediator of immune responses, inflammation, and sickness behaviors. Hormones and Behavior. 62 (3): 272-279.

Caro JF and Considine RV. 2006. Leptin: From laboratory to clinic. In: Handbook of obesity—clinical applications, George A Bray and Claude Bouchard editors. Marcel Dekker, Inc, New York.

Caro JF, Kolazynski JW, Nyce MR, et al. 1996a. Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanisms for leptin resistance. Lancet. 348(9021):159-61.

Caro JF, Sinha MK, Kolaczynski JW, Zhang PL. Considine RV. 1996b. Leptin: the tale of an obesity gene. Diabetes. 45(11): 1455-62.

Carulli L, Ferrari S, Bertolini M, Tagliafico E, and Del Rio G. 1999. Regulation of ob gene expression: evidence for epinephrine-induced suppression in human obesity. The Journal of clinical endocrinology and metabolism 84(9):3309-3312.

Carraro R, and Ruiz-Torres A. 2006. Relationship of serum leptin concentration with age, gender, and biomedical parameters in healthy, non-obese subjects. Archives of gerontology and geriatrics 43(3):301-312.

Casabiell X, Pineiro V, Tome MA, et al. 1997. Presence of leptin in colostrums and/or breast milk from lactating mothers: a potential role in the regulation of neonatal food intake. J Clin Endocrinol Metabl. 82. 4270-4273.

Casabiell X, Pineiro V, Peino R, Lage M, Camina J, Gallego R, Vallejo LG, Dieguez C, and Casanueva FF. 1998. Gender differences in both spontaneous and stimulated leptin secretion by human omental adipose tissue in vitro: dexamethasone and estradiol stimulate leptin release in women, but not in men. The Journal of clinical endocrinology and metabolism 83(6):2149-2155

Castracane VD, Kraemer GR, Ogden BW, and Kraemer RR. 2006. Interrelationships of serum estradiol, estrone, and estrone sulfate, adiposity, biochemical bone markers, and leptin in post-menopausal women. Maturitas 53(2):217-225.

Chan JL, Heist K, DePaoli AM, Veldhuis JD, and Mantzoros CS. 2003. The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. The Journal of clinical investigation 111(9):1409-1421.

Chan JL, Wong SL, and Mantzoros CS. 2008. Pharmacokinetics of subcutaneous recombinant methionyl human leptin administration in healthy subjects in the fed and fasting states: regulation by gender and adiposity. Clinical pharmacokinetics 47(11):753-764.

Chan JL, Matarese G, Shetty GK, Raciti P, et al. 2006. Differential regulation of metabolic, neuroendocrine, and immune function by leptin in humans. Proceedings of the National Academy of Science. 103 (22): 8481-8486.

Chan JL, Bullen J, Stoyneva V, et al. 2005. Recombinant Methionyl human leptin administration to achieve high physiological or pharmacologic leptin levels does not alter circulating inflammatory marker levels in humans with leptin sufficiency or excess. The Journal of Clinical Endocrinology & Metabolism. 90: 1618-1624.

Chan JL, Moschos SJ, Bullen J, et al. 2005b. Recombinant Methionyl Human leptin administration activates signal transducer and activator of transcription 3 signaling in peripheral blood mononuclear cells in Vivo and regulates soluble tumor necrosis factor – α receptor levels in humans with relative leptin deficiency. Journal of clinical endocrinology & metabolism. 1625-1631.

Chan JL, Heist K, DePaoli AN, Veldhuis JD, Mantzoros CS. 2003. The role of falling leptin levels in the neuro-endocrine and metabolic adaptation to short-term starvation in healthy men. The Journal of Clinical Investigation. 111: 1409-1421.

Chappell LC, Seed PT, Briley A, et al. 2002. A longitudinal study of biochemical variables in women at risk of preeclampsia. Obstet Gynecol. 187: 127-36.

Chardonnens D, Cameo P, Aubert ML, et al. 1999. Modulation of human cytotrophoblastic leptin secretion by interleukin-1α and 17β-oestradiol and its effect on HCG secretion. Molecular Human Reproduction. 5 (11): 1077-1082.

Chanprasertyothin S, Piaseu N, Chailurkit L, Rajatanavin R, and Ongphiphadhanakul B. 2005. Association of circulating leptin with bone mineral density in males and females. Journal of the Medical Association of Thailand = Chotmaihet thangphaet 88(5):655-659.

Chehab FF, Qiu J, Ogus S. 2004. The use of animal models to dissect the biology of leptin. Recent Prog Horm Res. 59:245-66.

Chehab FF, Qiu J, Mounzih K, Ewart-Toland A, Ogus S. 2002. Leptin and reproduction. Nutr Rev. 60 (10 Pt 2): S39-46.

Chehab FF. 1997. The reproductive side of leptin. Nature Medicine. 952-953.

Chehab FF, Lim ME, Lu R. 1996. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. Nature Genetics. 12: 318-320.

Chessler SD, Fujimoto WY, Shofer JB, Boyko EJ, and Weigle DS. 1998. Increased plasma leptin levels are associated with fat accumulation in Japanese Americans. Diabetes 47(2):239 243.

Chicchon A. 2005. Faunal resource use by the Chimane of Eastern Bolivia. In. Sponsel LE. Editor. Indigenous Peoples and the Future of Amazonia. Tuscan: University of Arizona Press. 225-243.

Chicchón A. 1992. Chimane resource use and market involvement in the Beni Biosphere Reserve, Bolivia. Ph.D. dissertation. University of Florida, Gainesville.

Chicurel M. 2000. Whatever happened to leptin. 404 (6778): 538-40.

Chin-Chance C, Polonsky KS, Schoeller DA. 2000. Twenty-Four-Hour Leptin Levels Respond to Cumulative Short-Term Energy Imbalance and Predict Subsequent Intake. The Journal of Clinical Endocrinology and Metabolism. 85: 2685-2691.

Christensen JO, Svendsen OL, Hassager C, and Christiansen C. 1998. Leptin in overweight postmenopausal women: no relationship with metabolic syndrome X or effect of exercise in addition to diet. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 22(3):195-199.

Chun WK, Belfi K, Melvin C, et al. 1998. Heterozygosity for Lepob or Leprdb affects body composition and leptin homeostasis in adult mice. American Journal of Physiology – Regulatory, integrative, and comparative physiology. 274: R985-R990.

Clement K, Vaisse C, Lahlou N, et al. 1998. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature. 392 (6674): 398-401.

Coleman DL. 1973. Effects of parabiosis of obese with diabetes and normal mice. Diabetologia. 9(4):294-8.

Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL et al. . 1996. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. The New England journal of medicine 334(5):292-295.

Considine RV, Premkumar A, Reynolds JC, Sebring NG, Ricks M, and Sumner AE. 2008. Adiponectin and leptin in African Americans. Obesity (Silver Spring) 16(2):428-434.

Cook T and Auwerx J. 2003. Leptin: cutting the fat off the bone. Lancet. 362: 1572-74.

Cooper JA, Polonsky KS, and Schoeller DA. 2009. Serum Leptin Levels in Obese Males During Over- and Underfeeding. Obesity 17(12):2149-2154.

Correia ML, Haynes WG, Rahmouni K, et al. 2002. The concept of selective leptin resistance: evidence from agouti yellow obese mice. 51(2):439-42.

Couillard C, Mauriege P, Prud'homme D, Nadeau A, Tremblay A, Bouchard C, and Despres JP. 1997. Plasma leptin concentrations: gender differences and associations with metabolic risk factors for cardiovascular disease. Diabetologia 40(10):1178-1184.

Couillard C, Mauriege P, Prud'homme D, Nadeau A, Tremblay A, Bouchard C, and Despres JP. 2002. Plasma leptin response to an epinephrine infusion in lean and obese women. Obesity research 10(1):6-13.

Crimmins EM, and Seeman TE. 2004. Integrating biology into the study of health disparities. Popul Dev Rev 30:89-107.

Cseh K, Baranyi E, Melczer Z, et al. 2002. The pathophysiological influence of leptin and the tumor nexrosis factor system on maternal insulin resistance: negative correlation with anthropometric parameters of neonates in gestational diabetes. Gynecol Endocrinol. 16: 453-460.

D'Adamo M, Buongiorno A, Maroccia E, Leonetti F, Barbetti F, Giaccari A, Zorretta D, Tamburrano G, and Sbraccia P. 1998. Increased OB gene expression leads to elevated plasma leptin concentrations in patients with chronic primary hyperinsulinemia. Diabetes 47(10):1625-1629.

Dagogo-Jack S, Fanelli C, Paramore D, Brothers J, and Landt M. 1996. Plasma leptin and insulin relationships in obese and nonobese humans. Diabetes 45(5):695-698.

Dagogo-Jack S, Selke G, Melson AK, and Newcomer JW. 1997. Robust leptin secretory responses to dexamethasone in obese subjects. The Journal of clinical endocrinology and metabolism 82(10):3230-3233.

Dammann G, Dierkes J, Graf M, Wiesbeck GA, Pridzun L, Schulte T, Westphal S, Luley C, Allen JP, and Wurst FM. 2005. No significant effect of acute moderate alcohol intake on leptin levels in healthy male volunteers. Addiction biology 10(4):357-364.

Davis MM. 2008. A Perscription For Human Immunology. Immunity. 29 (6): 835-838.

Dallongeville J, Hecquet B, Lebel P, Edme JL, Le Fur C, Fruchart JC, Auwerx J, and Romon M. 1998. Short term response of circulating leptin to feeding and fasting in man: influence of circadian cycle. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 22(8):728-733.

de Graaf C, Blom WAM, Smeets PAM, Stafleu A, Hendriks HFJ. 2004. Biomarkers of statiation and satiety. American Journal of Clinical Nutrition. 79: 946-61.

de Luis DA, Aller R, Izaola O, Gonzalez Sagrado M, Bellioo D, Conde R. 2007. Effects of a low-fat versus a low-carbohjydrate diet on adipocytokines in obese adults. Hormone Research. 67(6): 296-300.

Demir B, Guven S, Guven ESG, et al. 2007. Serum leptin level in women with unexplained infertility. Journal of Reproductive Immunology. 145-149.

Denevan WM. 1966. The Aboriginal Cultural Geography of the Llanos de Moxos of Bolivia. La Paz, Bolivia: University of California Press.

DeRosa V, Procaccini C, Cali G, et al. 2007. A key role of leptin in the control of regulatory T Cell Proliferation. Immunity. 26: 241-255.

Desgorces FD, Chennaoui M, Gomez-Merino D, Drogou C, and Guezennec CY. 2004. Leptin response to acute prolonged exercise after training in rowers. European journal of applied physiology 91(5-6):677-681.

Desgorces FD, Chennaoui M, Gomez-Merino D, Drogou C, Bonneau D, and Guezennec CY. 2004. Leptin, catecholamines and free fatty acids related to reduced recovery delays after training. European journal of applied physiology 93(1-2):153-158.

Deurenberg P, Deurenberg-Yap, Guricci S. 2002. Asians are different from Caucasians and from each other in their body mass index/body fat per cent relationships. Obesity Reviews. 3: 141-146.

Deurenberg P, Weststrate JA, and Seidell JC. 1991. Body mass index as a measure of body fatness: age- and sex-specific prediction formulas. The British journal of nutrition 65(2):105-114.

De Waart FG, Ruowei L, Deurenberg P. 1993. Comparison of body composition assessments by bioelectrical impedance and by anthropometry in premenopausal Chinese women. British Journal of Nutrition. 69: 657-664.

Dirlewanger M, di Vetta V, Guenat E, Battilana P, Seematter G, Schneiter P, Jequier E, and Tappy L. 2000. Effects of short-term carbohydrate or fat overfeeding on energy expenditure and plasma leptin concentrations in healthy female subjects. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 24(11):1413-1418.

Dirlewanger M, Di Vetta V, Giusti V, Schneiter P, Jequier E, and Tappy L. 1999. Effect of moderate physical activity on plasma leptin concentration in humans. European journal of applied physiology and occupational physiology 79(4):331-335.

Donahoo WT, Jensen DR, Yost TJ, and Eckel RH. 1997. Isoproterenol and somatostatin decrease plasma leptin in humans: a novel mechanism regulating leptin secretion. The Journal of clinical endocrinology and metabolism 82(12):4139-4143.

Donahue RP, Zimmet P, Bean JA, Decourten M, DeCarlo Donahue RA, Collier G, Goldberg RB, Prineas RJ, Skyler J, and Schneiderman N. 1999. Cigarette smoking, alcohol use, and physical activity in relation to serum leptin levels in a multiethnic population: The Miami Community Health Study. Annals of epidemiology 9(2):108-113.

Doucet E, Pomerleau M, Harper M-E. 2004. Fasting and Postprandial Total Ghrelin Remain Unchanged after Short-Term Energy Restriction. Journal of Clinical Endocrinology and Metabolism. 89: 1727-1732.

Douchi T, Iwamoto I, Yoshimitsu N, Ohishi Y, and Nagata Y. 2002. Differences in leptin production by regional fat mass in postmenopausal women. Endocrine journal 49(4):413-416.

Douchi T, Iwamoto I, Yoshimitsu N, Kosha S, and Nagata Y. 2002. Leptin production in pre- and postmenopausal women. Maturitas 42(3):219-223.

Duclos M, Corcuff JB, Ruffie A, Roger P, and Manier G. 1999. Rapid leptin decrease in immediate post-exercise recovery. Clinical endocrinology 50(3):337-342.

Durnin, J.V.G.A. and Womersley J. 1974. Body fat assessed from total body density and its estimation from skinfold thisckness: measeurements on 481 men and women aged from 15 to 72 years. Bristish Journal of nutrition 32:77-97.

Eaton SB, Pike MC, Short RV, et al. 1994. Women's Reproductive Cancers In Evolutionary Context. The Quarterly Review of Biology. 69(3): 353-367.

Eckert ED, Pomeroy C, Raymond N, et al. 1998. Leptin in anorexia nervosa. J Clin Endocrinol Metab. 83(3): 791-5.

Elbers JM, Asscheman H, Seidell JC, Frolich M, Meinders AE, and Gooren LJ. 1997. Reversal of the sex difference in serum leptin levels upon cross-sex hormone administration in transsexuals. The Journal of clinical endocrinology and metabolism 82(10):3267-3270.

Ellison PT. 1994. Salivary Steroids and Natural Variation in Human Ovarian Function. Annals of the New York Academy of Sciences. 709: 287-298.

Elefteriou F, Takeda S, Ebihara K, et al. 2004. Serum leptin level is a regulator of bone mass. PNAS. 101(9): 3258-3262.

El-Haschimi K and Lehnert H. 2003. Leptin resistance – or why leptin fails to work in obesity. Exp Clin Endocrinol Diabetes. 111(1): 2-7.

Elias AN, Pandian MR, Wang L, Suarez E, James N, and Wilson AF. 2000. Leptin and IGF-I levels in unconditioned male volunteers after short-term exercise. Psychoneuroendocrinology 25(5):453-461.

Elimam A, Knutsson U, Bronnegard M, Stierna P, Albertsson-Wikland K, and Marcus C. 1998. Variations in glucocorticoid levels within the physiological range affect plasma leptin levels. European journal of endocrinology / European Federation of Endocrine Societies 139(6):615-620.

Ellis R. 1996. A Taste for Movement: An Exploration of the Social Ethics of the Tsimane' of Lowland Bolivia. Ph.D. dissertation, St. Andrews University, Scotland.

Echwald SM, Clausen JO, Hansen T, Urhammer SA, Hansen L, Dinesen B, Borch-Johnsen, and Pedersen O. 1999. Analysis of the relationship between fasting serum leptin levels and estimates of beta-cell function and insulin sensitivity in a population sample of 380 healthy young Caucasians. European Journal of Endocrinology. 140: 180-185.

Erickson CL. 2001. Precolumbian Fish Farming in the Amazon. Expedition. 43(1):7-9.

Erickson CL. 2008. Amazonia: The Historical Ecology of a Domesticated Landscape. In. Handbook of South American Archaeology, edited by Helaine Silverman and William H. Isbell. Springer, New York, pg 157-189.

Essig DA, Alderson NL, Ferguson MA, Bartoli WP, and Durstine JL. 2000. Delayed effects of exercise on the plasma leptin concentration. Metabolism: clinical and experimental 49(3):395-399.

Eston RG, Fu F, Fung L. 1995. Validity of convential anthropometric techniques for predicting body composition in healthy Chinese adults. British Journal of Sports Medicine. 29(1): 52-56.

Evans K, Clark ML, and Frayn KN. 2001. Carbohydrate and fat have different effects on plasma leptin concentrations and adipose tissue leptin production. Clin Sci (Lond) 100(5):493-498.

Farooqi IS and O'Rahilly S. 2009. Leptin: a pivotal regulator of human energy homeostasis. Am J Clin Nutr. 89: 980S-4S.

Farooqi IS, Wangensteen T, Collins S, et al. 2007. Clinical and Molecular Genetic Spectrum of Congenital Deficiency of the Leptin Receptor. The New England Journal of Medicine. 356 (3): 237-247.

Farooqi IS, Matarese G, Lord GM, et al. 2002. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. 110: 1093-1103.

Farooqi IS, Jebb SA, Langmack G, et al. 1999. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. N Engl J Med. 341(12): 879-84.

Fatouros IG, Tournis S, Leontsini D, Jamurtas AZ, Sxina M, Thomakos P, Manousaki M, Douroudos I, Taxildaris K, and Mitrakou A. 2005. Leptin and adiponectin responses in overweight inactive elderly following resistance training and detraining are intensity related. The Journal of clinical endocrinology and metabolism 90(11):5970-5977.

Fernandez JR, Heo M, Heymsfield SB, Pierson RN, Jr., Pi-Sunyer FX, Wang ZM, Wang J, Hayes M, Allison DB, and Gallagher D. 2003. Is percentage body fat differentially related to body mass index in Hispanic Americans, African Americans, and European Americans? The American journal of clinical nutrition 77(1):71-75.

Festa A, Shnawa N, Krugluger W, et al. 1999. Relative hypoleptinaemia in women with mild gestational diabetes mellitus. Diabetic Medicine. 16: 656-662.

Fietta P. 2005. Focus on leptin, a pleiotropic hormone. Minerva Medica. 96(2): 65-75.

Filip R, and Raszewski G. 2009. Bone mineral density and bone turnover in relation to serum leptin, alpha-ketoglutarate and sex steroids in overweight and obese postmenopausal women. Clin Endocrinol (Oxf) 70(2):214-220.

Filozof CM, Murua C, Sanchez MP, Brailovsky C, Perman M, Gonzalez CD, and Ravussin E. 2000. Low plasma leptin concentration and low rates of fat oxidation in weight-stable post-obese subjects. Obesity research 8(3):205-210.

Fisher JS, Van Pelt RE, Zinder O, Landt M, and Kohrt WM. 2001. Acute exercise effect on postabsorptive serum leptin. J Appl Physiol 91(2):680-686.

Fitscher BA, Riedel HD, Young KC, and Stremmel W. 1998. Tissue distribution and cDNA cloning of a human fatty acid transport protein (hsFATP4). Biochimica et biophysica acta 1443(3):381-385.

Flier JS. 1998. What's in a name? In search of leptin's physiologic role. The journal of clinical endocrinology & metabolism. 83(5): 1407-1413.

Fogteloo J, Meinders E, Frolich M, McCamish M, and Pijl H. 2003. The decline in plasma leptin in response to calorie restriction predicts the effects of adjunctive leptin treatment on body weight in humans. European journal of internal medicine 14(7):415-418.

Forsum E, Sadurskis A, Wager J. 1989. Estimation of body fat in healthy Swedish women during pregnancy and lactation. American Journal of Clinical Nutrition. 465-73.

Foster Z, Byron E, Reyes-Garcia V, Huanca T, Vadez V, Apaza L, Perez E, Tanner S, Gutierrez Y, Sandstrom B, Yakhedts A, Osburn C, Godoy R, Leonard WR. 2005. Physical growth and nutritional status of Tsimane Amerindian children of lowland Bolivia. American Journal of Physical Anthropology 126:343-51.

Franks PW, Loos RJ, Brage S, O'Rahilly S, Wareham NJ, and Ekelund U. 2007. Physical activity energy expenditure may mediate the relationship between plasma leptin levels and worsening insulin resistance independently of adiposity. J Appl Physiol 102(5):1921-1926.

Frederich R, Hu S, Raymond N, and Pomeroy C. 2002. Leptin in anorexia nervosa and bulimia nervosa: importance of assay technique and method of interpretation. The Journal of laboratory and clinical medicine 139(2):72-79.

Freeman AC, Yousafzai AK, Filteau SM, Pai M. 2002. Serum leptin in disabled and non-disabled children in an Indian slum population. Eur J Clin Nutr. 56(10): 967-72.

Friedman JM. 2009. Leptin at 14 y of age: an ongoing story. American Journal of clinical nutrition. 89(3): 973S-979S.

Friedman JM. 2002. The function of leptin in nutrition, weight, and physiology. Nutr Rev. 60 (10 Pt 2): S1-14.

Friedman JM, and Halaas JL. 1998. Leptin and the regulation of body weight in mammals. Nature 395(6704):763-770.

Frisancho R. 1993. Human Adaptation and Accommodation. University of Michigan Press. Ann Arbor.

Fruehwald-Schultes B, Oltmanns KM, Kern W, Born J, Fehm HL, Peters A. 2002. The effect of experimentally induced insulin resistance on the leptin response to hyperinsulinaemia. International Journal of Obesity. 26: 510-516.

Garofalo C and Surmacz E. 2006. Leptin and Cancer. Journal of Cell Physiology. 207: 12-22.

Gavrilova O, Barr V, Marcus-Samuels B, et al. 1997. Hyperleptinemia of Pregnancy Associated with the Appearance of a Circulating form of the leptin receptor. The Journal of Biological Chemistry. 272(48). 30546-30551.

Gettler LT, McDade TW, Agustin SS, Kuzawa CW. 2011. Short-term changes in fathers' hormones during father-child play: Impacts of paternal attitudes and experience. Hormones and Behavior. 60: 599-606

Gibson RS. 2005. Principles of Nutritional Assessment, 2nd edition. Oxford University Press.

Gippini A, Mato A, Peino R, Lage M, Dieguez C, and Casanueva FF. 1999. Effect of resistance exercise (body building) training on serum leptin levels in young men. Implications for relationship between body mass index and serum leptin. Journal of endocrinological investigation 22(11):824-828.

Godoy R, Goodman E, Reyes-Garcia V, Eisenberg DTA, Leonard WR, Huanca T, McDade TW, Tanner S, and Jha N. 2007. Climate and Child Height among Native Amazonians. Tsimane' Amazonian Panel Study Working Paper. Boston. p 24.

Godoy R, Goodman E, Reyes-Garcia V, Eisenberg DT, Leonard WR, Huanca T, McDade TW, Tanner S, and Jha N. 2008. Rain, temperature, and child-adolescent height among Native Amazonians in Bolivia. Annals of human biology 35(3):276-293.

Godoy R, Byron E, Reyes-Garcia V, Vadez V, Leonard WR, Apaza L, Huanca T, Perez E, and Wilkie D. 2005. Income inequality and adult nutritional status: anthropometric evidence from a pre-industrial society in the Bolivian Amazon. Soc Sci Med 61(5):907-919.

Godoy RA, Leonard WR, Reyes-Garcia V, Goodman E, McDade T, Huanca T, Tanner S, and Vadez V. 2006. Physical stature of adult Tsimane' Amerindians, Bolivian Amazon in the 20th century. Economics and human biology 4(2):184-205.

Godoy R. 2001. Indians, markets, and rainforests: theoretical, comparative, and quantitative explorations in the neotropics. New York: Columbia University Press.

Godoy R, Kirby K, and Wilkie D. 2001. Tenure security, private time preference, and use of natural resources among lowland Bolivian Amerindians. Ecological Economics 38:105-118.

Godoy R, Magvanjav O, Nyberg C, Eisenberg DT, McDade TW, Leonard WR, Reyes-Garcia V, Huanca T, Tanner S, and Gravlee C. 2010. Why no adult stunting penalty or height premium? Estimates from native Amazonians in Bolivia. Economics and human biology 8(1):88-99.

Gomez-Merino D, Chennaoui M, Drogou C, Bonneau D, and Guezennec CY. 2002. Decrease in serum leptin after prolonged physical activity in men. Medicine and science in sports and exercise 34(10):1594-1599.

Goulding A, and Taylor RW. 1998. Plasma leptin values in relation to bone mass and density and to dynamic biochemical markers of bone resorption and formation in postmenopausal women. Calcified Tissue Int 63(6):456-458.

Grattan DR, Ladyman SR, Augustine RA. 2007. Hormonal induction of leptin resistance during pregnancy. Physiol Behav. 91(4): 366-74.

Grinspoon SK, Askari H, Landt ML, Nathan DM, Schoenfeld DA, Hayden DL, Laposata M, Hubbard J, and Klibanski A. 1997. Effects of fasting and glucose infusion on basal and overnight leptin concentrations in normal-weight women. The American journal of clinical nutrition 66(6):1352-1356.

Groschl M, Wagner R, Dorr HG, Blum W, Rascher W, and Dotsch J. 2000. Variability of leptin values measured from different sample matrices. Hormone research 54(1):26-31.

Groschl M, Knerr I, Topf HG, Schmid P, Rascher W, and Rauh M. 2003. Endocrine responses to the oral ingestion of a physiological dose of essential amino acids in humans. The Journal of endocrinology 179(2):237-244.

Grosfeld A, Andre J, Hauguel-De Mouson S, et al. 2002. Hypoxia-inducible factor 1 transactivates the human leptin gene promoter. J Biol Chem. 277(45): 42953-7.

Gualillo O, Gonzalez-Juanatey JR, and Lago F. 2007. The emerging role of adipokines as mediators of cardiovascular function: Physiologic and clinical perspectives. Trends Cardiovas Med 17(8):275-283.

Guerra B, Fuentes T, Delgado-Guerra S, Guadalupe-Grau A, Olmedillas H, Santana A, Ponce-Gonzalez JG, Dorado C, and Calbet JA. 2008. Gender dimorphism in skeletal muscle leptin receptors, serum leptin and insulin sensitivity. PloS one 3(10):e3466.

Gurven MD. 2000. How can we distinguish between mutational "hot spots" and "old sites" in human mtDNA samples? Human Biology. 72(3):455-471.

Gurven M, Kaplan H, Winking J, Eid Rodriguez D, Vasunilashorn S, Kim JK, Finch C, and Crimmins E. 2009. Inflammation and infection do not promote arterial aging and cardiovascular disease risk factors among lean horticulturalists. PloS one 4(8):e6590.

Haffner SM, Miettinen H, Mykkanen L, and Stern MP. 1998. Leptin concentrations are associated with higher proinsulin and insulin concentrations but a lower proinsulin/insulin ratio in non-diabetic subjects. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 22(9):899-905.

Hagobian TA, Sharoff CG, Stephens BR, Wade GN, Silva JE, Chipkin SR, and Braun B. 2009. Effects of exercise on energy-regulating hormones and appetite in men and women. American journal of physiology Regulatory, integrative and comparative physiology 296(2):R233-242.

Halaas JL, Boozer C, Blair-West J, et al. 1997. Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. Proceedings of the National Academy of Science. 94: 8878-8883.

Halaas JL, Gajiwala KS, Maffei M, et al. 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. Science. 269 (5223): 543-6.

Hamilton BS, Paglia D, Kwan AY, Deitel M. 1995. Increased obese mRNA expression in omental fat cells from massively obese humans. 1(9): 953-6.

Hamrick MW. 2004. Leptin, bone mass, and the thrifty phenotype. J Bone Miner Res 19(10):1607-1611.

Harrold JA. 2004. Leptin leads hypothalamic feeding circuits in a new direction. Bioessays. 26(10): 1043-5.

Hardie L, Trayhurn P, Abramovich D, and Fowler P. 1997. Circulating leptin in women: a longitudinal study in the menstrual cycle and during pregnancy. Clinical Endocrinology. 47: 101-106.

Hasbum B, Real JT, Sanchez C, Priego MA, Diaz J, Viguer A, Basanta M, Martinez-Valls J, Marin J, Carmena R et al. . 2006. Effects of a controlled program of moderate physical exercise on insulin sensitivity in nonobese, nondiabetic subjects. Clinical journal of sport medicine : official journal of the Canadian Academy of Sport Medicine 16(1):46-50.

Haugen F, Ranheim T, Narsem NK, et al. 2006. Increased plasma levels of adipokines in preeclampsia: relationship to placenta and adipose tissue gene expression. Am J Physiol Endocrinol Metab. 290: E326-E333.

Havel PJ, Kasim-Karakas S, Dubuc GR, Mueller W, and Phinney SD. 1996. Gender differences in plasma leptin concentrations. Nature medicine 2(9):949-950.

Havel PJ, Townsend R, Chaump L, and Teff K. 1999. High-fat meals reduce 24-h circulating leptin concentrations in women. Diabetes 48(2):334-341.

Hayday AC and Peakman M. 2008. The habitual, diverse and surmountable obstacles to human immunology research. 9 (6): 575-580.

Haynes WG. 2005. Role of leptin in obesity-related hypertension. Exp Physiol. 90(5): 683-8.

Heckenberger M and Neves NG. 2009. Amazonian archaeology. Annual Review of Anthropology. 38:251-266.

Heini AF, Lara-Castro C, Kirk KA, et al. 1998. Association of leptin and hunger-satiety ratings in obese women. International Journal of Obesity. 22: 1084-1087.

Hellstrom L, Wahrenberg H, Hruska K, Reynisdottir S, and Arner P. 2000. Mechanisms behind gender differences in circulating leptin levels. Journal of internal medicine 247(4):457-462.

Henrich J, Heine SJ, Norenzayan A. 2010. The weirdest people in the world? Behavioral and Brain Sciences. 33 (2/3): 1-75.

Hendler I, Blackwell SC, Mehta SH, et al. 2005. The levels of leptin, adiponectin, and resistin in normal weight, overweight, and obese pregnant women with and without preeclampsia. American Journal of Obstetrics and Gynecology. 193: 979-83.

Henson MC and Castracane VD. 2006. Leptin in Pregnancy: An Update. Biology of Reproduction. 74. 218-229.

Henson MC and Castacane VD. 2000. Leptin in Pregnancy. Biology of Reproduction. 63. 1219-1228.

Hervey GR. 1959. The effects of lesions in the hypothalamus in parabiotic rats. 145 (2): 336-52.

Heymsfield SB, Greenberg AS, Fujioka K, et al. 1999. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. JAMA. 282(16): 1568-75.

Hickey MS, and Calsbeek DJ. 2001. Plasma leptin and exercise: recent findings. Sports Med 31(8):583-589.

Hickey MS, Considine RV, Israel RG, Mahar TL, McCammon MR, Tyndall GL, Houmard JA, and Caro JF. 1996. Leptin is related to body fat content in male distance runners. The American journal of physiology 271(5 Pt 1):E938-940.

Hickey MS, Houmard JA, Considine RV, Tyndall GL, Midgette JB, Gavigan KE, Weidner ML, McCammon MR, Israel RG, and Caro JF. 1997. Gender-dependent effects of exercise training on serum leptin levels in humans. The American journal of physiology 272(4 Pt 1):E562-566.

Highman TJ, Friedman JE, Huston LP, et al. 1998. Longitudinal changes in maternal serum leptin concentrations, body composition, and resting metabolic rate in pregnancy. Am J Obstet Gynecol. 178: 1010-1015.

Hilton LK, and Loucks AB. 2000. Low energy availability, not exercise stress, suppresses the diurnal rhythm of leptin in healthy young women. American journal of physiology Endocrinology and metabolism 278(1):E43-49.

Himms-Hagen J. 1999. Physiological roles of the leptin endocrine system: differences between mice and humans. Critical Reviews in Clinical Laboratory Sciences. 36(6): 575-655.

Himms-Hagen J. 1997. On raising energy expenditure in ob/ob mice. Science 276(5315):1132-1133.

Ho SC, Tai ES, Eng PH, Ramli A, Tan CE, and Fok AC. 1999. A study in the relationships between leptin, insulin, and body fat in Asian subjects. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 23(3):246-252.

Hodge AM, de Courten MP, Dowse GK, Zimmet PZ, Collier GR, Gareeboo H, Chitson P, Fareed D, Hemraj F, Alberti KG et al. . 1998. Do leptin levels predict weight gain?--A 5-year follow-up study in Mauritius. Mauritius Non-communicable Disease Study Group. Obesity research 6(5):319-325.

Hodge AM, Westerman RA, de Courten MP, Collier GR, Zimmet PZ, and Alberti KG. 1997. Is leptin sensitivity the link between smoking cessation and weight gain? International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 21(1):50-53.

Hong SC, Yoo SW, Cho GJ, Kim T, Hur JY, Park YK, Lee KW, and Kim SH. 2007. Correlation between estrogens and serum adipocytokines in premenopausal and postmenopausal women. Menopause 14(5):835-840.

Hortobagyi T, Israel RG, and O'Brien KF. 1994. Sensitivity and specificity of the Quetelet index to assess obesity in men and women. European journal of clinical nutrition 48(5):369-375.

Houmard JA, Cox JH, MacLean PS, and Barakat HA. 2000. Effect of short-term exercise training on leptin and insulin action. Metabolism: clinical and experimental 49(7):858-861.

Howard JK, Cave BJ, Oksanen LJ, et al. 2004. Enhanced leptin sensitivity and attenuation of diet-induced obesity in mice with haploinsufficiency of Socs3. Nat Med. 10(7): 734-8.

Huanca T*:* 1999. Tsimane' Indigenous Knowledge. Swidden Fallow Management and Conservation. Ph. D. thesis. University of Florida.

Huang L, Wang Z, Li C. 2001. Modulation of circulating leptin levels by its soluble receptor. J Biol Chem. 276(9):6343-9.

Hube F, Lietz U, Igel M, Jensen PB, Tornqvist H, Joost HG, Hauner H. 1996. Difference in leptin mRNA levels between omental and subcutaneous abdominal adipose tissue from obese humans. Hormone and Metabolic Research. 28(12): 690-3.

Huckshorn CJ, Westerterp-Plantenga MS, Saris WHM. 2003. Pegylated human recombinant leptin (PEG-OB) causes additional weight loss in severely energy-restricted, overweight men. Am J Clin Nutr. 77: 771-6.

Hukshorn CJ,Saris WHM,Westerterp Plantenga MS,Farid AR, Smith FJ,Campfield LA. 2000. Weekly subcutaneous pegylated recombi-nant native human leptin (PEG-OB) administration in obese men. J Clin Endocrinol Metab. 85:4003–9.

Hukshorn CJ, and Saris WH. 2004. Leptin and energy expenditure. Current opinion in clinical nutrition and metabolic care 7(6):629-633.

Iftikhar U, Khoja A, Iqbal MA, et al. 2008. Evaluation of serum leptin levels during normal pregnancy and in preeclampsia. J Ayub Med Coll Abbottabad. 20(4): 137-140.

Iglesias MJ, Eiras S, Pineiro R, Lopez-Otero D, Gallego R, Fernandez AL, Lago F, and Gonzalez-Juanatey JR. 2006. [Gender differences in adiponectin and leptin expression in epicardial and subcutaneous adipose tissue. Findings in patients undergoing cardiac surgery]. Revista espanola de cardiologia 59(12):1252-1260.

Ingalls AM, Dickie MM, Snell GD. 1950. Obese, a new mutation in the house mouse. Journal of heredity. 41(12): 317-8.

Ioffe E, Moon B, Connolly E, and Friedman JM. 1998. Abnormal regulation of the leptin gene in the pathogenesis of obesity. Proceedings of the National Academy of Sciences of the United States of America 95(20):11852-11857.

Iputo JE, Robinson D, and Mguni M. 2001. Serum leptin concentration in a rural African population. East African medical journal 78(9):484-488.

Ishigaki T, Koyama K, Tsujita J, Tanaka N, Hori S, and Oku Y. 2005. Plasma leptin levels of elite endurance runners after heavy endurance training. Journal of physiological anthropology and applied human science 24(6):573-578.

James, P (moderator). 2002. Discussion. Nutrition Reviews. 60(10): S68-S84.

Janik JE, Curti BD, Considine RV, Rager HC, Powers GC, Alvord WG, Smith JW, 2nd, Gause BL, and Kopp WC. 1997. Interleukin 1 alpha increases serum leptin concentrations in humans. The Journal of clinical endocrinology and metabolism 82(9):3084-3086.

Jasienski M, Bazzaz FA. 1999. The Fallacy of Ratios and the Testability of Models in Biology. Oikos. 84. 321-326.

Jebb SA, Siervo M, Fruhbeck G, Goldberg GR, Mugatroyd PR, Prentice AM. 2006. Variability of appetite control mechanism in response to 9 weeks of progressive overfeeding in humans. International Journal of Obesity. 1160-1162.

Jenkins AB, and Campbell LV. 2003. Does relative leptinemia predict weight gain in humans? Obesity research 11(3):373-374.

Jenkins AB, Markovic TP, Fleury A, and Campbell LV. 1997. Carbohydrate intake and short-term regulation of leptin in humans. Diabetologia 40(3):348-351.

Jensen MD, Hensrud D, O'Brien PC, and Nielsen S. 1999. Collection and interpretation of plasma leptin concentration data in humans. Obesity research 7(3):241-245.

Jeon JY, Steadward RD, Wheeler GD, Bell G, McCargar L, and Harber V. 2003. Intact sympathetic nervous system is required for leptin effects on resting metabolic rate in people with spinal cord injury. The Journal of clinical endocrinology and metabolism 88(1):402-407.

Joannic J, Oppert J, Lahlou N, et al. 1998. Plasma Leptin and Hunger Ratings in Healthy Humans. Appetite. 30: 129-138.

Jockenhovel F, Blum WF, Vogel E, Englaro P, Muller-Wieland D, Reinwein D, Rascher W, and Krone W. 1997. Testosterone substitution normalizes elevated serum leptin levels in hypogonadal men. The Journal of clinical endocrinology and metabolism 82(8):2510-2513.

Johnston FE and Little MA. 2000. History of Human Biology in the United States of America. In: Human Biology: An Evolutionary and Biocultural Perspective. Editors S. Stinson, B. Bogin, R. Huss-Ashmore, DH. O'Rouke, 27- 46. New York: Wiley, John & Sons, Incorporated. Pgs 656.

Johnstone AM, Murison SD, Duncan JS, Rance KA, and Speakman JR. 2005. Factors Influencing variation in basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but not sex, circulating leptin, or triiodothyronine. The American journal of clinical nutrition 82(5):941-948.

Jones JC. 1980. Conflict between Whites and Indians on the Llanos de Moxos, Beni Department: A Case Study in Development from the Cattle Regions of the Bolivian Oriente. Ph.D dissertation, Department of Anthropology, University of Florida. Gainesville, FL.

Jorgensen JO, Vahl N, Dall R, and Christiansen JS. 1998. Resting metabolic rate in healthy adults: relation to growth hormone status and leptin levels. Metabolism: clinical and experimental 47(9):1134-1139.

Jurimae J, and Jurimae T. 2006. Influence of insulin-like growth factor-1 and leptin on bone mineral content in healthy premenopausal women. Exp Biol Med (Maywood) 231(10):1673-1677.

Jurimae J, Purge P, and Jurimae T. 2005. Adiponectin is altered after maximal exercise in highly trained male rowers. European journal of applied physiology 93(4):502-505.

Jurimae J, and Jurimae T. 2006. Influence of insulin-like growth factor-1 and leptin on bone mineral content in healthy premenopausal women. Exp Biol Med (Maywood) 231(10):1673-1677.

Jurimae J, Maestu J, and Jurimae T. 2003. Leptin as a marker of training stress in highly trained male rowers? European journal of applied physiology 90(5-6):533-538.

Jurimae J, Purge P, and Jurimae T. 2007. Effect of prolonged training period on plasma adiponectin in elite male rowers. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 39(7):519-523.

Kafulafula GE, Moodley J, Ojwang PF, Kagoro H. 2002. Leptin and pre-eclampsia in black African parturients. BJOG: an international journal of obstetrics and gynaecology. 109: 1256-1261.

Kalhan R, Puthawala K, Agarwal S, Amini SB, and Kalhan SC. 2001. Altered lipid profile, leptin, insulin, and anthropometry in offspring of South Asian immigrants in the United States. Metabolism: clinical and experimental 50(10):1197-1202.

Kaplowitz PB. 2008. Link Between Body Fat and the Timing of Puberty. Pediatrics. 121: S208-S217.

Karamouzis I, Karamouzis M, Vrabas IS, Christoulas K, Kyriazis N, Giannoulis E, and Mandroukas K. 2002. The effects of marathon swimming on serum leptin and plasma neuropeptide Y levels. Clinical chemistry and laboratory medicine : CCLM / FESCC 40(2):132-136.

Karhunen L, Haffner S, Lappalainen R, et al. 1997. Serum leptin and short-term regulation of energy in obese women. Clinical Science. 92 (6): 573-578.

Kastin AJ, Pan W, Maness et al. 1999. Decreased transport of leptin across the blood-brain barrier in rats lacking the short form of the leptin receptor. Peptides. 20(12): 1449-53.

Kawai M. Devlin MJ, Rosen R. 2009. Fat targets for skeletal health. Nat. Rev. Rheumatol. 5: 365–372.

Kaufman BA, Warren MP, Dominguez JE, Wang J, Heymsfield SB, and Pierson RN. 2002. Bone density and amenorrhea in ballet dancers are related to a decreased resting metabolic rate and lower leptin levels. J Clin Endocr Metab 87(6):2777-2783.

Kautzky-Willer A, Pacini G, Tura A, et al. 2001. Increased plasma leptin in gestational diabetes. Diabetologia. 44:164-172.

Keim NL, Stern JS, and Havel PJ. 1998. Relation between circulating leptin concentrations and appetite during a prolonged, moderate energy deficit in women. American Journal of Clinical Nutrition. 68: 794-801.

Keller P, Keller C, Steensberg A, Robinson LE, and Pedersen BK. 2005. Leptin gene expression and systemic levels in healthy men: effect of exercise, carbohydrate, interleukin-6, and epinephrine. J Appl Physiol 98(5):1805-1812.

Kennedy A, Gettys TW, Watson P, Wallace P, Ganaway E, Pan Q, and Garvey WT. 1997. The metabolic significance of leptin in humans: gender-based differences in relationship to adiposity, insulin sensitivity, and energy expenditure. The Journal of clinical endocrinology and metabolism 82(4):1293-1300.

Kettaneh A, Heude B, Romon M, Oppert JM, Borys JM, Balkau B, Ducimetiere P, and Charles MA. 2007. High plasma leptin predicts an increase in subcutaneous adiposity in children and adults. European journal of clinical nutrition 61(6):719-726.

Kiess W, Schubring C, Prohaska F, et al. 1997. Leptin levels in Amniotic Fluid, Cord blood, and maternal serum at term and in amniotic fluid and maternal serum at midgestation. In: Leptin, the voice of adipose tissue. Editors: W.F Blum, W Kiess, and W. Rascher, 192-197. Heidelberg-Leipzig: JA Barth Verlag. 333 pg.

Kim SM, Kim SH, Lee JR, Jee BC, Ku SY, Suh CS, Choi YM, Kim JG, and Moon SY. 2008. Association of leptin receptor polymorphisms Lys109Arg and Gln223Arg with serum leptin profile and bone mineral density in Korean women. American journal of obstetrics and gynecology 198(4):421 e421-428.

Klein S, Coppack SW, Mohamed-Ali V, Landt M. 1996. Adipose Tissue Leptin Production and Plasma Leptin Kinetics in Humans. Diabetes. 45: 984-987.

Klein S, Horowitz JF, Landt M, Goodrick SJ, Mohamed-Ali V, and Coppack SW. 2000. Leptin production during early starvation in lean and obese women. American journal of physiology Endocrinology and metabolism 278(2):E280-284.

Klein LC, Corwin EJ, and Ceballos RM. 2004. Leptin, hunger, and body weight: Influence of gender, tobacco smoking, and smoking abstinence. Addictive behaviors 29(5):921-927.

Klok MD, Jakobsdottir S, and Drent ML. 2007. The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. 8: 21-34.

Knerr I, Groschl M, Rascher W, and Rauh M. 2003. Endocrine effects of food intake: insulin, ghrelin, and leptin responses to a single bolus of essential amino acids in humans. Annals of nutrition & metabolism 47(6):312-318.

Kocyigit Y, Atamer Y, Atamer A, et al. 2004. Changes in serum levels of leptin, cytokines and lipoprotein in preeclamptic and normotensive pregnant women. Gynecol Endocrinol. 19: 267-273.

Koistinen HA, Tuominen JA, Ebeling P, Heiman ML, Stephens TW, and Koivisto VA. 1998. The effect of exercise on leptin concentration in healthy men and in type 1 diabetic patients. Medicine and science in sports and exercise 30(6):805-810.

Kolaczynski JW, Ohannesian JP, Considine RV, et al. 1996a. Response of leptin to short-term and prolonged overfeeding in humans. J Clin Endocrinol Metab. 81(11):4162-5.

Kolaczynski JW, Considine RV, Ohannesian J, et al. 1996b. Response of leptin to short-term fasting and refeeding in humans: a link with ketogenesis but not ketones themselves. 45(11): 1511-5.

Koistinen HA, Koivisto VA, Andersson S, et al. 1997. Leptin Concentration in Cord Blood Correlates with Intrauterine Growth. Journal of Clinical Endocrinology and Metabolism. 82(10): 3328-3330.

Kolaczynski EW, Goldstein BJ, Considine RV. 1997. Dexamethasone, OB Gene, and Leptin in Humans; Effect of Exogenous Hyperinsulinemia. Journal of Clinical Endocrinology and Metabolism 82(11):3895-3897.

Kopecký J, Krušinová E, Klementová M, Kazdová L, Mlejnek P, Pravenec M, Hill M, Pelikánová1 T. 2010 Selected adipokines - plasma concentrations and adipose tissue expressions during 24-hour lipid infusion in healthy men. Physiological Research. 59(1): 89-96.

Korbonits M, Trainer PJ, Little JA, Edwards R, Kopelman PG, Besser GM, Svec F, and Grossman AB. 1997. Leptin levels do not change acutely with food administration in normal or obese subjects, but are negatively correlated with pituitary-adrenal activity. Clinical endocrinology 46(6):751-757.

Koutkia P, Canavan B, Johnson ML, DePaoli A, and Grinspoon S. 2003. Characterization of leptin pulse dynamics and relationship to fat mass, growth hormone, cortisol, and insulin. American journal of physiology Endocrinology and metabolism 285(2):E372-379.

Kosaki A, Yamada K, and Kuzuya H. 1996. Reduced expression of the leptin gene (ob) by catecholamine through a G(S) protein-coupled pathway in 3T3-L1 adipocytes. Diabetes 45(12):1744-1749.

Kraemer RR, Chu H, and Castracane VD. 2002. Leptin and exercise. Exp Biol Med (Maywood) 227(9):701-708.

Kristensen K, Pedersen SB, and Richelsen B. 2000. Interactions between sex steroid hormones and leptin in women. Studies in vivo and in vitro. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 24(11):1438-1444.

Krizova J, Eretova V, Haluzikova J, et al. 2004. Soluble leptin receptor and leptin levels in pregnant women before and after delivery. Endocrine Research. 30(3): 379-385.

Kuchroo VK, Nicholson LB. 2003. Fast and feel good. Nature. 422: 27-28.

Kutoh E, Boss O, Levasseur F, Giacobino J-P. 1998. Quantification of the full length receptor (OB-Rb) in human brown and white adipose tissue. 62(5): 445-451.

Kuzawa CW, Quinn EA, and Adair LS. 2007. Leptin in a lean population of Filipino adolescents. American journal of physical anthropology 132(4):642-649.

Kuzawa CW, Hallal PC, Adair L, et al. 2012. Birth Weight, Postnatal Weight Gain, and Adult Body Composition in Five Low and Middle Income Countries. American Journal of Human Biology. 24: 5-13.

Kyriakakou M, Malamitsi-Puchner A, Militsi H, et al. 2008. Leptin and adiponectin concentrations in intrauterine growth restricted and appropriate for gestational age fetuses, neonates, and their mothers. European Journal of Endocrinology. 158 (3): 343-8.

Kyriazis GA, Caplan JD, Lowndes J, Carpenter RL, Dennis KE, Sivo SA, and Angelopoulos TJ. 2007. Moderate exercise-induced energy expenditure does not alter leptin levels in sedentary obese men. Clinical journal of sport medicine : official journal of the Canadian Academy of Sport Medicine 17(1):49-51.

Lage M, Garcia-Mayor RV, Tome MA, et al. 1999. Serum leptin levels in women throughout pregnancy and the postpartum period and in women suffering spontaneous abortion. Clinical Endocrinology. 50: 211-216.

Laivuori H, Kaaja R, Koistinen H, et al. 2000. Leptin during and after preeclamptic or normal pregnancy: its relation to serum insulin and insulin sensitivity. Metabolism. 49(2): 259-263.

Laird SM, Quinton ND, Anstie B, et al. 2001. Leptin and leptin-binding activity in women with recurrent miscarriage: correlation with pregnancy outcome. Human reproduction. 16(9): 2008-2013.

Lam QLK and Lu L. 2007. Role of Leptin in Immunity. Cellular & Molecular Immunology. 4(1): 1-13.

Lambrinoudaki I, Christodoulakos G, Panoulis C, Botsis D, Rizos D, Augoulea A, and Creatsas G. 2003. Determinants of serum leptin levels in healthy postmenopausal women. Journal of endocrinological investigation 26(12):1225-1230.

Laml T, Preyer O, Hartmann BW, et al. 2001. Decreased maternal serum leptin in pregnancies complicated by preeclampsia. Reproductive sciences. 8:89-93.

Lammert A, Kiess W, Bottner A, et al. 2001. Soluble leptin receptor represents the main leptin binding activity in human blood. Biochem Biophys Res Commun. 283(4): 982-8.

Landt M, Lawson GM, Helgeson JM, Davila-Roman VG, Ladenson JH, Jaffe AS, and Hickner RC. 1997. Prolonged exercise decreases serum leptin concentrations. Metabolism: clinical and experimental 46(10):1109-1112.

Langenberg C, Bergstrom J, Laughlin GA, and Barrett-Connor E. 2005. Ghrelin, adiponectin, and leptin do not predict long-term changes in weight and body mass index in older adults: longitudinal analysis of the Rancho Bernardo cohort. American journal of epidemiology 162(12):1189-1197.

Larsson H, Elmstahl S, and Ahren B. 1996. Plasma leptin levels correlate to islet function independently of body fat in postmenopausal women. Diabetes 45(11):1580-1584.

Lasker GW. 1969. Human Biological Adaptability. Science. 166: 1480-1486.

Lasley BL, Mobed K, Gold EB. 1994. The Use of Urinary Hormonal Assessments in Human Studies. Annals of the New York Academy of Sciences. 709: 299-311.

Laughlin GA, Yen SS. 1997. Hypoleptimia in women athletes: absence of a diurnal rhythm with amenorrhea. Journal of Clinical Endocrinology and Metabolism 82: 318-21.

Lavens D, Montoye T, Piessevaux J, et al. 2006. A complex interaction pattern of CIS and SOCS2 with the leptin receptor. Journal of Cell Science. 119 (11): 2214-2224.

Leal-Cerro A, Garcia-Luna PP, Astorga R, Parejo J, Peino R, Dieguez C, and Casanueva FF. 1998. Serum leptin levels in male marathon athletes before and after the marathon run. The Journal of clinical endocrinology and metabolism 83(7):2376-2379.

Leavens DA, Bard KA and Hopkins WD. 2010. BIZARRE chimpanzees do not represent "the chimpanzee" Behavioral and Brain Sciences. 33 (2/3): 26-27.

Lederman, SA. 2005. Pregnancy. In: Human Body Composition, editors Heymsfield SB, Lohman TG, Wang Z. Human Kinetics: 299-311.

Lee GH, Proenca R, Montez JM, et al. 1996. Abnormal splicing of the leptin receptor in diabetic mice. Nature. 379 (8566): 632-5.

Lee MJ, Wang Y, Ricci MR, Sullivan S, Russell CD, and Fried SK. 2007. Acute and chronic regulation of leptin synthesis, storage, and secretion by insulin and dexamethasone in human adipose tissue. American journal of physiology Endocrinology and metabolism 292(3):E858-864.

Legakis IN, Mantzouridis T, Saramantis A, and Lakka-Papadodima E. 2004. Rapid decrease of leptin in middle-aged sedentary individuals after 20 minutes of vigorous exercise with early recovery after the termination of the test. Journal of endocrinological investigation 27(2):117-120.

Leonard WR, Sorensen MV, Mosher MJ, Spitsyn V, and Comuzzie AG. 2009. Reduced fat oxidation and obesity risks among the Buryat of Southern Siberia. American journal of human biology : the official journal of the Human Biology Council 21(5):664-670.

Leonard WR and Katzmarzyk PT. 2010. Body size and shape: climatic and nutritional influences on human body morphology. In: Muehlenbein MP, editor. Human Evolutionary Biology. Cambridge, UK: Cambridge University Press. p 157-169.

Lepercq J, Guerre-Millo M, Andre J, et al. 2003. Leptin: a potential marker of placental insufficiency. Gynecologic and Obstetric Investigation. 55: 151-155.

Lepercq J, Challier J-C, Guerre-Millo M, et al. 2001. Prenatal leptin production: evidence that fetal adipose tissue produces leptin. The Journal of Clinical Endocrinology and Metabolism. 86(6): 2409-2413.

Lepercq J, Cauzac M, Lahlou N, et al. 1998. Overexpression of placental leptin in diabetic pregnancy: a critical role for insulin. Diabetes. 47: 847-850.

Levine JA, Eberhardt NL, and Jensen MD. 1999. Leptin responses to overfeeding: relationship with body fat and nonexercise activity thermogenesis. The Journal of clinical endocrinology and metabolism 84(8):2751-2754.

Levine JA, Eberhardt NL, and Jensen MD. 1999. Role of nonexercise activity thermogenesis in resistance to fat gain in humans. Science 283(5399):212-214.

Lewandowski K, Horn R, O'Callaghan et al. 1999. Free Leptin, Bound Leptin, and Soluble Leptin Receptor in Normal and Diabetic Pregnancies. Journal of Clinical Endocrinology and Metabolism. 84(1): 300-306.

Li RHW, Poon SCS, Yu MY, Wong YF. 2004. Expression of Placental Leptin and Leptin Receptors in Preeclampsia. International Journal of Gynecological Pathology. 23: 378-385.

Liao SC, Lee MB, Lee YJ, and Huang TS. 2004. Hyperleptinemia in subjects with persistent partial posttraumatic stress disorder after a major earthquake. Psychosomatic medicine 66(1):23-28.

Licinio J, Negrao AB, Mantzoros C, Kaklamani V, Wong ML, Bongiorno PB, Mulla A, Cearnal L, Veldhuis JD, Flier JS et al. . 1998. Synchronicity of frequently-sampled 24-hour concentrations of circulating leptin, luteinizing hormone, and estradiol in healthy women. J Invest Med 46(3):220A-220A.

Licinio J, Mantzoros C, Negrao AB, Cizza G, Wong ML, Bongiorno PB, Chrousos GP, Karp B, Allen C, Flier JS et al. . 1997. Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. Nature medicine 3(5):575-579.

Lin KC, Sagawa N, Yura S, Itoh H, and Fujii S. 2005. Simultaneous increases of leptin and gonadotropin-releasing hormone following exogenous estrogen administration in women with normally menstrual cycle. Endocrine journal 52(4):449-454.

Lindgarde F, Widen I, Gebb M, and Ahren B. 2004. Traditional versus agricultural lifestyle among Shuar women of the Ecuadorian Amazon: effects on leptin levels. Metabolism: clinical and experimental 53(10):1355-1358.

Lindgarde F, Ercilla MB, Correa LR, and Ahren B. 2004. Body adiposity, insulin, and leptin in subgroups of Peruvian Amerindians. High altitude medicine & biology 5(1):27-31.

Lindroos AK, Lissner L, Carlsson B, Carlsson LM, Torgerson J, Karlsson C, Stenlof K, and Sjostrom L. 1998. Familial predisposition for obesity may modify the predictive value of serum leptin concentrations for long-term weight change in obese women. The American journal of clinical nutrition 67(6):1119-1123.

Linnemann K, Malek A, Schneider H, Fusch C. 2001. Physiological and pathological regulation of feto/placenta/maternal leptin expression. Biochemical Society Transactions. 29(2): 86-90.

Linnemann K, Malek A, Sager R, et al. 2000. Leptin Production and Release in the Dually in Vitro Perfused Human Placenta. 85(11): 4298-4301.

Little MA and Kennedy KAR. 2010. Introduction to the History of American Physical Anthropology. In: Histories of American Physical Anthropology in the Twentieth Century.

Lock M. 2013. The Epigenome and Nature/Nurture Reunification: A Challenge for Anthropology. Medical Anthropology: Cross-Cultural Studies in Health and Illness. Accepted.

Lock M and Kaufert P. 2001. Menopause, Local Biologies, and Cultures of Aging. American Journal of Human Biology. 13: 494-504.

Loffler S, Aust G, Kohler U, Spanel-Borowski K. 2001. Evidence of leptin expression in normal and polycystic human ovaries. Molecular Human Reproduction. 7 (12): 1143-1149.

Lohman TG, Roche AF, and Martorell R. 1988. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Books. vi, 177 p. p.

Lopes IM, Forga L, and Martinez JA. 2001. Effects of leptin resistance on acute fuel metabolism after a high carbohydrate load in lean and overweight young men. Journal of the American College of Nutrition 20(6):643-648.

Lord G. 2002. Role of leptin in immunology. Nutrition Reviews. 60 (10 Pt 2): S35-8.

Lord GM, Matarese G, Howard JK, et al. 1998. Leptin modulates the T-cell immune response and reverses starvation-induced immunesuppression. Nature. 394: 897-901.

Lorentzon M, Landin K, Mellstrom D, and Ohlsson C. 2006. Leptin is a negative independent predictor of areal BMD and cortical bone size in young adult Swedish men. J Bone Miner Res 21(12):1871-1878.

Lohman TG, Roche AF, Martorell R. 1988. Anthropometric Standardization Reference Manual. Human Kinetics Pub.

Loucks AB. 2007. Energy availability and infertility. Current Opinion in Endocrinology, Diabetes & Obesity. 14: 470-474.

Luo JD, Zhang GS, Chen MS. 2005. Leptin and cardiovascular disease. Drug News Perspectives. 18(7): 427-31.

Lu D, Yang X, Wu Y. et al. 2006. Serum adiponectin, leptin and soluble leptin receptor in pre-eclampsia. International Journal of Gynecology and Obstetrics. 95: 121-126.

Luke AH, Rotimi CN, Cooper RS, Long AE, Forrester TE, Wilks R, Bennett FI, Ogunbiyi O, Compton JA, and Bowsher RR. 1998. Leptin and body composition of Nigerians, Jamaicans, and US blacks. The American journal of clinical nutrition 67(3):391-396.

Lutoslawska G, Skierska E, and Byszerwska-Szpocinska E. 2004. Plasma leptin concentrations in lean, physically active males and females in relation to body composition and metabolic variables. The Journal of sports medicine and physical fitness 44(2):200-206.

Luukkaa V, Pesonen U, Huhtaniemi I, Lehtonen A, Tilvis R, Tuomilehto J, Koulu M, and Huupponen R. 1998. Inverse correlation between serum testosterone and leptin in men. The Journal of clinical endocrinology and metabolism 83(9):3243-3246.

Ma Z, Gingerich RL, Santiago JV, Klein S, Smith CH, and Landt M. 1996. Radioimmunoassay of leptin in human plasma. Clinical chemistry 42(6 Pt 1):942-946,

Mackintosh RM, and Hirsch J. 2001. The effects of leptin administration in non-obese human subjects. Obesity research 9(8):462-469.

Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S et al. . 1995. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nature medicine 1(11):1155 1161.

Maguire R, McMillan AM, McArdle C. 2002. A longitudinal study of lepin and appetite, resting energy expenditure and body fat mass in weight-stable cancer patients. Cytokine. 20 (4): 174-7.

Mainardia M, Scabiab G, Vottarib T, Santinic F, Pincherac A, Maffeia L, Pizzorussod T,and Maffeib M. 2010. A sensitive period for environmental regulation of eating behavior and leptin sensitivity. Pnas. 1073. 1-6.

Malmstrom R, Taskinen MR, Karonen SL, and Yki-Jarvinen H. 1996. Insulin increases plasma leptin concentrations in normal subjects and patients with NIDDM. Diabetologia 39(8):993-996.

Mantzoros CS, Flier JS. 2000. Editorial: leptin as a therapeutic agent-trials and tribulations. J Clin Endocrinol Metab. 85(11): 4000-2.

Mantzoros CS, Moschos S, Avramopoulos I, Kaklamani V, Liolios A, Doulgerakis DE, Griveas I, Katsilambros N, and Flier JS. 1997. Leptin concentrations in relation to body mass index and the tumor necrosis factor-alpha system in humans. J Clin Endocr Metab 82(10):3408-3413.

Mantzoros CS, Liolios AD, Tritos NA, Kaklamani VG, Doulgerakis DE, Griveas I, Moses AC, and Flier JS. 1998. Circulating insulin concentrations, smoking, and alcohol intake are important independent predictors of leptin in young healthy men. Obesity research 6(3):179-186.

Margetic S. Gazzola C, Pegg GG, Hill RA. 2002. Leptin: a review of its peripheral actions and interactions. International Journal of Obesity. 26: 1407-1433.

Mark AL, Correia ML, Rahmouni K, Haynes WG. 2002. Selective leptin resistance: a new concept in leptin physiology with cardiovascular implications. J. Hypertens. 20(7): 1245-50.

Markowska A, Malendowicz K, and Drews K. 2004. The role of leptin in breast cancer. European Journal of Gynaecological Oncology. 25(2): 192-194.

Marques-Vidal P, Bochud M, Paccaud F, Mooser V, Waeber G, and Vollenweider P. 2010. Distribution of plasma levels of adiponectin and leptin in an adult Caucasian population. Clinical endocrinology 72(1):38-46.

Mars M, de Graaf C, de Groot C, et al. 2006. Fasting leptin and appetite responses induced by a 4-day 65%-energy-restricted diet. International Journal of Obesity. 30: 122-128.

Mars M, de Graaf C, de Goot L CPGM, Kok FJ. 2005. Decreases in fasting leptin and insulin concentrations after acute energy restriction and subsequent compensation in food intake. 81: 570-7.

Marshall JA, Grunwald GK, Donahoo WT, Scarbro S, and Shetterly SM. 2000. Percent body fat and lean mass explain the gender difference in leptin: analysis and interpretation of leptin in Hispanic and non-Hispanic white adults. Obesity research 8(8):543-552.

Martin LJ, Mahaney MC, Almasy L, Hixson JE, Cole SA, MacCluer JW, Jaquish CE, Blangero J, and Comuzzie AG. 2002. A quantitative trait locus on chromosome 22 for serum leptin levels adjusted for serum testosterone. Obesity research 10(7):602-607.

Martinez-Abundis E, Gonzalez-Ortiz M, Pascoe-Gonzalez S. 2000. Serum leptin levels and the severity of preeclampsia. Arch Gynecol Obstet. 264(2): 71-73.

Martini G, Valenti R, Giovani S, Campagna S, Franci B, and Nuti R. 2001. Leptin and body composition in healthy postmenopausal women. Panminerva Med 43(3):149-154.

Martin-Romero C, Santos-Alvarez J, Goberna R, Sanchez-Margalet V. 2000. Human Leptin Enhances Activation and Proliferation of Human Circulating T Lymphocytes. Cellular Immunology. 199: 15-24.

Masuzaki H, Ogawa Y, Sagawa N, et al. 1997. Nonadipose tissue production of leptin: Leptin as a novel placenta-derived hormone in humans. 3(9): 1029-1033.

Masuzaki H, Ogawa Y, Hosoda K, Miyawaki T, Hanaoka I, Hiraoka J, Yasuno A, Nishimura H, Yoshimasa Y, Nishi S et al. . 1997. Glucocorticoid regulation of leptin synthesis and secretion in humans: elevated plasma leptin levels in Cushing's syndrome. The Journal of clinical endocrinology and metabolism 82(8):2542-2547.

Matarese G, Moschos S, Mantzoros CS. 2005. Leptin in Immunology. The journal of Immunology. 173: 3137-3142.

Matarese G, La Cava A, Sana V, et al. 2002. Balancing susceptibility to infection and autoimmunity: a role for leptin. Trends in Immunology. 23 (4): 182-187.

Mavri A, Stegnar M, and Sabovic M. 2001. Do baseline serum leptin levels predict weight regain after dieting in obese women? Diabetes, obesity & metabolism 3(4):293-296.

Mestas J and Hughes CCW. 2004. Of Mice and Not Men: Differences between Mouse and Human Immunology. The Journal of Immunology. 172: 2731-2738.

McCarthy EA, Strauss BJC, Walker SP, and Permezel M. 2004. Determination of Maternal Body Composition in Pregnancy and Its Relevance to Perinatal Outcomes. Obstetrical and Gynecological Survey. 59 (10): 731-742.

McCarthy JF, Misra DN, Roberts JM. 1999. Maternal plasma leptin is increases in preeclampsia and positively correlates with fetal cord concentration. Am J Obstet Gynecol. 180:731-6.

McDade TW. 2003. Life history theory and the immune system: steps toward a human ecological immunology. Yearbookd of Physical Anthropology. 46: 100-125.

McDade TW, Leonard WR, Burhop J, Reyes-García V, Vadez V, Huanca T, and Godoy R. 2003. Acculturation, C-reactive protein, and child growth in lowland Bolivia. American Journal of Human Biology. 15:273-274.

McDade TW, Williams S, Snodgrass JJ. 2007. What a drop can do: Dried blood spots as a minimally invasive method for integrating biomarkers into population-based research. Demography. 44(4): 899-925.

McDade TW. 2005. Life history, maintenance, and the early origins of immune function. Am J Hum Biol 17(1):81-94.

McDade TW, Leonard WR, Burhop J, Reyes-Garcia V, Vadez V, Huanca T, and Godoy RA. 2005. Predictors of C-Reactive Protein in Tsimane' 2 to 15 years-old in Lowland Bolivia. American Journal of Physical Anthropology 128:906-913.

McDade TW and Shell-Duncan B. 2005. Whole blood collected on filter paper provides a minimally-invasive method for assessing human transferring receptor. Journal of Nutrition. 132: 3760-3763.

McDade TW, Stallings JF, Angold A, Costello EJ, Burleson M, Cacioppo JT, et al. 2000. Epstein-Barr virus antibodies in whole blood spots: a minimally-invasive method for assessing an aspect of cell-mediated immunity. Psychosomatic Medicine. 62:560-567.

McLachlan KA, O'Neal D, Jenkins A, Alford FP. 2006. Do adiponectin, TNFα, leptin and CRP relate to insulin resistance in pregnancy? Studies in women with and without gestational diabetes, during and after pregnancy. Diabetes Metab Res Rev. 22: 131-138.

Messinis IE, Papageorgiou I, Milingos S, Asprodini E, Kollios G, and Seferiadis K. 2001. Oestradiol plus progesterone treatment increases serum leptin concentrations in normal women. Hum Reprod 16(9):1827-1832.

Metraux A. 1942. Native tribes of Eastern Boliva and Motto Grasso. Washington: US government print-off.

Metraux A. 1963. Tribes of the eastern slopes of the Bolivian Andes. In. Steard JH Editor. Handbook of SouthAmerican Indians. New York:Cooper Square Publishers, Inc. 465-506.

Miell JP, Englaro P, and Blum WF. 1996. Dexamethasone induces an acute and sustained rise in circulating leptin levels in normal human subjects. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 28(12):704-707.

Miller AA, Sharrock KCB, McDade TW. 2006. Measurement of Leptin in Dried Blood Spot Samples. American Journal of Human Biology. 18(6): 857-860.

Miller DW and Ballor DL. 1989. Validity of Anthropometric Percent Fat Prediction Equations for use with Pregnant Women. American Journal of Human Biology. 1: 451-455. Mukherjea 1999

Mise H, Yura S, Itoh H, et al. 2007. The relationship between Maternal Plasma Leptin Levels and Fetal Growth Restriction. Endocrine Journal. 54(6): 945-951.

Mise H, Norimasa S, Matsumoto T, et al. 1998. Augmented placental production of leptin in preeclampsia: possible involvement of placental hypoxia. Journal of clinical endocrinology and metabolism. 83: 3225-3229.

Montague CT, Prins JB, Sanders L, Digby JE, and O'Rahilly S. 1997. Depot- and sex-specific differences in human leptin mRNA expression: implications for the control of regional fat distribution. Diabetes 46(3):342-347.

Montague CT, Farooqi IS, Whitehead JP, et al. 1997. Congenital leptin deficiency is associated with severly early-onset obesity in humans. Nature. 387(6636): 903-8.

Morberg CM, Tetens I, Black E, Toubro S, Soerensen TIA, Pedersen O, and Astrup A. 2003. Leptin and bone mineral density: A cross-sectional study in obese and nonobese men. J Clin Endocr Metab 88(12):5795-5800.

Mori H, Hanada R, Hanada T, et al. 2004. Socs3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet-induced obesity. Nat Med. 10(7): 739-43.

Muscelli E, Camastra S, Masoni A, Baldi S, Sironi AM, Natali A, and Ferrannini E. 1996. Acute insulin administration does not affect plasma leptin levels in lean or obese subjects. European journal of clinical investigation 26(10):940-943.

Mouzon SH, Lepercq J, Catalano P. 2006. The known and unknown of leptin in pregnancy. American Journal of Obstetrics and Gynecology. 194: 1537-45.

Muller TD, Focker M, Holtkamp K, et al. 2008. Leptin-mediated neuroendocrine alterations in Anorexia Nervosa: Somatic and Behavioral Implications. Child and adolescent psychiatric clinics of North America. 18(1): 117-129.

Mullington JM, Chan JL, Van Dongen HPA, Szuba MP, Samaras J, Price NJ, Meier-Ewert HK, Dinges DF, and Mantzoros CS. 2003. Sleep loss reduces diurnal rhythm amplitude of leptin in healthy men. J Neuroendocrinol 15(9):851-854.

Mukherjea R, Castonguay TW, Douglass LW, Moser-Veillon P. 1999. Elevated Leptin Concentrations in Pregnancy and Lactation: Possible Role As a Modulator of Substrate Utilization. Life Sciences. 65(11): 1183-1193.

Mumtaz F, Memon AR, Yousfani S, et al. 2008. Role of serum leptin level as a marker of severity of preeclampsia. J Ayub Med Coll Abbottabad. 20(1): 13-15.

Munzberg H, Flier JS, Bjorbaek C. 2004. Region-specific leptin resistance within the hypothalamus of diet-induced obese mice. 145(11): 4880-9.

Munzberg H and Myers MG Jr. 2005. Molecular and anatomical determinants of central leptin resistance. Nat Neurosci. 8(5): 566-70

Myers MG, Cowley MA, Munzberg H. 2008. Mechanisms of leptin action and leptin resistance. Annu Rev Physiol. 70: 537-56.

Myers MG jr. 2004. Leptin receptor signaling and the regulation of mammalian physiology. Recent Prog Horm Res. 59: 287-304.

Nagy TR, Davies SL, Hunter GR, Darnell B, and Weinsier RL. 1998. Serum leptin concentrations and weight gain in postobese, postmenopausal women. Obesity research 6(4):257-261.

Nakatsukasa H, Masuyama H, Takamoto N, Hiramatsu Y. 2008. Circulating leptin and angiogenic factors in preeclampsia patients. Endocrine Journal. 55(3): 565-573.

Nar A, Demirtas E, Ayhan A, Gurlek A. 2009. Effects of bilateral ovariectomy and estrogen replacement therapy on serum leptin, sex hormone binding globulin and insulin like growth factor-1 levels. Gynecology and Endocrinology. 25(12): 773-8.

Neuhauser-Berthold M, Herbert BM, Luhrmann PM, Sultemeier AA, Blum WF, Frey J, and Hebebrand J. 2000. Resting metabolic rate, body composition, and serum leptin concentrations in a free-living elderly population. European journal of endocrinology / European Federation of Endocrine Societies 142(5):486-492.

Nicklas BJ, Toth MJ, and Poehlman ET. 1997. Daily energy expenditure is related to plasma leptin concentrations in older African-American women but not men. Diabetes 46(9):1389-1392.

Nies MA, Chen KY, and Vander Wal JS. 2004. Energy expenditure, body composition, and biochemical indicators in healthy community women. International journal of food sciences and nutrition 55(3):237-247.

Nindl BC, Scoville CR, Sheehan KM, Leone CD, and Mello RP. 2002. Gender differences in regional body composition and somatotrophic influences of IGF-I and leptin. J Appl Physiol 92(4):1611-1618.

Ning Y, Williams MA, Muy-Rivera M, et al. 2004. Relationship of maternal plasma leptin and risk of pre-eclampsia: a prospective study. The Journal of Maternal-Fetal and Neonatal Medicine. 186-192.

Niskanen LK, Haffner S, Karhunen LJ, Turpeinen AK, Miettinen H, and Uusitupa MI. 1997. Serum leptin in obesity is related to gender and body fat topography but does not predict successful weight loss. European journal of endocrinology / European Federation of Endocrine Societies 137(1):61-67.

Norgan NG. 1995. The assessment of the body composition of populations. In: Body composition techniques in health and disease. Editors Davies PSW and Cole TJ. Cambridge University Press. 195-221.

Nyberg C. 209. Market integration, stress, and health: an exploration of hypothalamic-pituitary-adrenal axis dynamics among the Tsimane' of the Bolivia Amazon. Dissertation. Northwestern University. Evanston, IL.

Odabasi E, Ozata M, Turan M, Bingol N, Yonem A, Cakir B, Kutlu M, and Ozdemir IC. 2000. Plasma leptin concentrations in postmenopausal women with osteoporosis. Eur J Endocrinol 142(2):170-173.

Ohannesian JP, Marco CC, Najm PS, Goldstein BJ, Caro JF, Kolaczynski JW. 1999. Small weight gain is not associated with development of insulin resistance in healthy, physically actove individuals. Hormone and metabolic research. 31(5): 323-5.

O'Leary VB, Marchetti CM, Krishnan RK, Stetzer BP, Gonzalez F, and Kirwan JP. 2006. Exercise-induced reversal of insulin resistance in obese elderly is associated with reduced visceral fat. J Appl Physiol 100(5):1584-1589.

Olive JL, and Miller GD. 2001. Differential effects of maximal- and moderate-intensity runs on plasma leptin in healthy trained subjects. Nutrition 17(5):365-369.

O'Rahilly S. 2002. Leptin: defining its role in humans by the clinical study of genetic disorders. Nutr Rev. 60 (10 pt 2): S30-4; discussion S68-84, 85-7.

O'Rahilly S. 1998. Life without leptin. Nature. 392 (6674): 330-1.

Ostlund RE, Jr., Yang JW, Klein S, and Gingerich R. 1996. Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. The Journal of clinical endocrinology and metabolism 81(11):3909-3913.

Otsuka R, Yatsuya H, Tamakoshi K, Matsushita K, Wada K, Toyoshima H. 2006. Perceived Psychological Stress and Serum Leptin Concentrations in Japanese Men. Obesity. 14(10): 1832-1838.

Ouyang Y, Chen H, Chen H. 2007. Reduced plasma adiponectin and elevated leptin in pre-eclampsia. International journal of gynecology and obstetrics. 98:110-114.

Ozata M, Ozdemir IC, Licinio J. 1999. Human leptin deficiency caused by a missense mutation: multiple endocrine defects, decreased sympathetic tone, and immune system dysfunction indicate new targets for leptin action, greater central than peripheral resistance to the effects of leptin, and spontaneous correction of leptin-mediated defects. Journals of Clinical Endocrinology and Metabolism. 84: 3686-3695.

Ozcelik O, Celik H, Ayar A, Serhatlioglu S, and Kelestimur H. 2004. Investigation of the influence of training status on the relationship between the acute exercise and serum leptin levels in obese females. Neuro endocrinology letters 25(5):381-385.

Palmert MR, Radovick S, Boepple PA. 1998. Leptin levels in children with central precocious puberty. Journal of clinical endocrinology and metab. 83(7): 2260-5.

Papaspyrou-Rao S, Schneider SH, Petersen RN, and Fried SK. 1997. Dexamethasone increases leptin expression in humans in vivo. The Journal of clinical endocrinology and metabolism 82(5):1635-1637.

Pasman WJ, Westerterp-Plantenga MS, and Saris WH. 1998. The effect of exercise training on leptin levels in obese males. The American journal of physiology. 274 (2 Pt 1):E280-286.

Patel AM, Godoy RA, Seyfried C, Reyes-Garcia V, Huanca T, Leonard WR, McDade TW, and Tanner S. 2007. On the accuracy of perceived parental height in a native Amazonian society. Economics and human biology 5(1):165-178.

Paxton A, Lederman SA, Heymsfield SB, Wang J, Thornton JC, and Pierson RN. 1998. Anthropometric equations for studying body fat in pregnant women. American Journal of Clinical Nutrition. 67: 104-110.

Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. 1995. Effects of the obese gene product on body weight regulation in ob/ob mice. Science. 269(5223):540-3.

Perfetto F, Tarquini R, Cornelissen G, Mello G, Tempestini A, Gaudiano P, Mancuso F, and Halberg F. 2004. Circadian phase difference of leptin in android versus gynoid obesity. Peptides 25(8):1297-1306.

Perkins KA, and Fonte C. 2002. Effects of smoking status and smoking cessation on leptin levels. Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco 4(4):459-466.

Peltz G, Sanderson M, Perez A, Sexton K, Ochoa Casares D, and Fadden MK. 2007. Serum leptin concentration, adiposity, and body fat distribution in Mexican-Americans. Archives of medical research 38(5):563-570.

Perusse L, Collier G, Gagnon J, Leon AS, Rao DC, Skinner JS, Wilmore JH, Nadeau A, Zimmet PZ, and Bouchard C. 1997. Acute and chronic effects of exercise on leptin levels in humans. J Appl Physiol 83(1):5-10.

Phillips DF. 1998. Leptin passes safety tests, but effectiveness varies. JAMA. 280(10): 869-70.

Pighetti M, Tommaselli GA, D'Elia A, et al. 2003. Maternal Serum and Umbilical Cord Blood Leptin Concentrations with fetal growth restriction. Obstet Gynecol. 102: 535-43.

Piland R. 1991. Traditional Tsimane' Agriculture and Its Relationship to Soils of the Beni Biosphere Reserve, Bolivia. M.A. thesis, University of Florida. Gainesville, FL.

Pineiro V, Casabiell X, Peino R, Lage M, Camina JP, Menendez C, Baltar J, Dieguez C, and Casanueva F. 1999. Dihydrotestosterone, stanozolol, androstenedione and dehydroepiandrosterone sulphate inhibit leptin secretion in female but not in male samples of omental adipose tissue in vitro: lack of effect of testosterone. The Journal of endocrinology 160(3):425-432.

Pinkney JH, Coppack SW, and Mohamed-Ali V. 1998. Effect of isoprenaline on plasma leptin and lipolysis in humans. Clinical endocrinology 48(4):407-411.

Plafker G. 1963. Observations on Archaeological Remains in Northeastern Bolivia. American Antiquity, 28(3): 372-378.

Popovic V, Micic D, Danjanovic S, Zoric S, Djurovic M, Obradovic S, Petakov M, Dieguez C, and Casanueva FF. 1998. Serum leptin and insulin concentrations in patients with insulinoma before and after surgery. European journal of endocrinology / European Federation of Endocrine Societies 138(1):86-88.

Poston L. 2002. Leptin and Preeclampsia. Seminars in Reproductive Medicine. 20(2): 131-138.

Pound P, Ebrahim S, Sandercock P, et al. 2004. Where is the evidence that animal research benefits humans? British Medical Journal. 328: 514-517.

Pratley RE, Nicolson M, Bogardus C, and Ravussin E. 1997. Plasma leptin responses to fasting in Pima Indians. The American journal of physiology 273(3 Pt 1):E644-649.

Pratley RE, Nicolson M, Bogardus C, and Ravussin E. 1996. Effects of acute hyperinsulinemia on plasma leptin concentrations in insulin-sensitive and insulin-resistant Pima Indians. The Journal of clinical endocrinology and metabolism 81(12):4418-4421.

Prentice AM, Moore SE, Collinson AC, and O'Connell MA. 2002. Leptin and Undernutrition. Nutrition Reviews. 60(10): S56-S67.

Puder JJ, Monaco SE, Sen Gupta S, Wang J, Ferin M, and Warren MP. 2006. Estrogen and exercise may be related to body fat distribution and leptin in young women. Fertility and sterility 86(3):694-699.

Quinn EA, Largado FE, Power M, and Kuzawa CW. 2012. Predictors of Breast Milk Macronutrient Composition in Filipino Mothers. American Journal of Human Biology. 24: 533-540.

Raben A, Agerholm-Larsen L, Flint A, Holst JJ, and Astrup A. 2003. Meals with similar energy densities but rich in protein, fat, carbohydrate, or alcohol have different effects on energy expenditure and substrate metabolism but not on appetite and energy intake. The American journal of clinical nutrition 77(1):91-100.

Racette SB, Coppack SW, Landt M, and Klein S. 1997. Leptin production during moderate-intensity aerobic exercise. The Journal of clinical endocrinology and metabolism 82(7):2275-2277.

Ramson R, Jurimae J, Jurimae T, and Maestu J. 2008. The influence of increased training volume on cytokines and ghrelin concentration in college level male rowers. European journal of applied physiology 104(5):839-846.

Rauch F, Blum WF, Klein K, Allolio B, and Schonau E. 1998. Does leptin have an effect on bone in adult women? Calcified Tissue Int 63(6):453-455.

Ravussin E, Pratley RE, Maffei M, Wang H, Friedman JM, Bennett PH, and Bogardus C. 1997. Relatively low plasma leptin concentrations precede weight gain in Pima Indians. Nature medicine 3(2):238-240.

Riester J. 1993. Universo mítico de los Chimanes. La Paz: Hisbol.

Reitman ML, Marcus-Samuels B, Gavrilova O. 2001. Leptin and its role in pregnancy and fetal development – an overview. Biochemical Society Transactions. 29(2): 68-72.

Rentsch J, Levens N, Chiesi M. Recombinant ob-gene product reduces food intake in fasted mice. Biochemistry and biophysical research communications. 214 (1). 131-6.

Reseland JE, Haugen F, Hollung K, et al. 2001. Reduction of leptin gene expression by dietary polyunsaturated fatty acids. Journal of Lipid Research. 42: 743-750.

Reseland JE, Syversen U, Bakke I, et al. 2001. Leptin is expressed in and Secreted from Primary Cultures of Human Osteoblasts and Promotes Bone Mineralization. Journal of Bone and Mineral Research. 16(8): 1426-1433.

Reseland JE, Anderssen SA, Solvoll K, Hjermann I, Urdal P, Holme I, and Drevon CA. 2001. Effect of long-term changes in diet and exercise on plasma leptin concentrations. The American journal of clinical nutrition 73(2):240-245.

Reseland JE, Mundal HH, Hollung K, Haugen F, Zahid N, Anderssen SA, and Drevon CA. 2005. Cigarette smoking may reduce plasma leptin concentration via catecholamines. Prostaglandins, leukotrienes, and essential fatty acids 73(1):43-49.

Reyes-García VE. 2001. Indigenous people, ethnobotanical knowledge, and market economy: a case study of the Tsimane' Amerindians in lowland Bolivia. Ph.D. dissertation. University of Florida. Gainesville. FL.

Reyes-Garcia V, McDade TW, Molina JL, Leonard WR, Tanner SN, Huanca T, and Godoy R. 2008. Social rank and adult male nutritional status: evidence of the social gradient in health from a foraging-farming society. Soc Sci Med 67(12):2107-2115.

Ribeiro R, Busnello JV, Licinio J. 2006. Pulsatile and Diurnal Leptin Rhythms. In: Castracane VD and Henson MC, editors. Leptin. New York: Springer. p 247-262.

Ricci MR, Fried SK, and Mittleman KD. 2000. Acute cold exposure decreases plasma leptin in women. Metabolism: clinical and experimental 49(4):421-423.

Ricci MR, Lee MJ, Russell CD, Wang Y, Sullivan S, Schneider SH, Brolin RE, and Fried SK. 2005. Isoproterenol decreases leptin release from rat and human adipose tissue through posttranscriptional mechanisms. American journal of physiology Endocrinology and metabolism 288(4):E798-804.

Rioja G. 1992. The Jatata Project: The Pilot Experience of Chimane Empowerment. *In* Sustainable Harvest and Marketing of Rain Forest Products. M. J. Plotkin and L, M. Famolare, eds. Pp. 192-196. Washington, DC: Island Press.

Rioja Ballivián G. 1996. Control Cultural e Identidad Tsimane'. Revista Museo Nacional de Etnografia y Folklore. 7-8:147-179. La Paz, Bolivia: Museo Nacional de Etnografía y Folklore.

Roberts SB, Nicholson M, Staten M, Dallal GE, Sawaya AL, Heyman MB, Fuss P, and Greenberg AS. 1997. Relationship between circulating leptin and energy expenditure in adult men and women aged 18 years to 81 years. Obesity research 5(5):459-463.

Rojdmark S, Calissendorff J, and Brismar K. 2001. Alcohol ingestion decreases both diurnal and nocturnal secretion of leptin in healthy individuals. Clinical endocrinology 55(5):639-647.

Rolland-Cachera MF, Maillot M, Deheeger M, Souberbielle JC, Peneau S, and Hercberg S. 2012. Association of nutrition in early life with body fat and serum leptin at adult age. Int J Obes (Lond).

Romon M, Lebel P, Velly C, et al. 1999. Leptin response to carbohydrate or fat meal association with subsequent satiety and energy intake. American Journal of Physiology, Endocrinology, and Metabolism. 277: 855-861.

Romon M, Lebel P, Fruchart JC, and Dallongeville J. 2003. Postprandial leptin response to carbohydrate and fat meals in obese women. Journal of the American College of Nutrition 22(3):247-251.

Rosen CJ, and Bouxsein ML. 2006. Mechanisms of disease: is osteoporosis the obesity of bone? Nat Clin Pract Rheum 2(1):35-43.

Rosenbaum M, Nicolson M, Hirsch J, Heymsfield SB, Gallagher D, Chu F, and Leibel RL. 1996. Effects of gender, body composition, and menopause on plasma concentrations of leptin. The Journal of clinical endocrinology and metabolism 81(9):3424-3427.

Rosenbaum M, Nicolson M, Hirsch J, Murphy E, Chu F, and Leibel RL. 1997. Effects of weight change on plasma leptin concentrations and energy expenditure. The Journal of clinical endocrinology and metabolism 82(11):3647-3654.

Rosenbaum M, and Leibel RL. 1999. Clinical review 107: Role of gonadal steroids in the sexual dimorphisms in body composition and circulating concentrations of leptin. The Journal of clinical endocrinology and metabolism 84(6):1784-1789.

Rosenbaum M, Pietrobelli A, Vasselli JR, Heymsfield SB, and Leibel RL. 2001. Sexual dimorphism in circulating leptin concentrations is not accounted for by differences in adipose tissue distribution. International journal of obesity and related metabolic

disorders : journal of the International Association for the Study of Obesity 25(9):1365-1371.

Rosenbaum M, Murphy EM, Heymsfield SB, Matthews DE, and Leibel RL. 2002. Low dose leptin administration reverses effects of sustained weight-reduction on energy expenditure and circulating concentrations of thyroid hormones. The Journal of clinical endocrinology and metabolism 87(5):2391-2394.

Rosenbaum M, Goldsmith R, Bloomfield D, Magnano A, Weimer L, Heymsfield S, Gallagher D, Mayer L, Murphy E, and Leibel RL. 2005. Low-dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight. The Journal of clinical investigation 115(12):3579-3586.

Rosenbaum M, Sy M, Pavlovich K, Leibel RL, and Hirsch J. 2008. Leptin reverses weight loss-induced changes in regional neural activity responses to visual food stimuli. The Journal of clinical investigation 118(7):2583-2591.

Roth MJ, Baer DJ, Albert PS, Castonguay TW, Dorgan JF, Dawsey SM, Brown ED, Hartman TJ, Campbell WS, Giffen CA et al. . 2003. Relationship between serum leptin levels and alcohol consumption in a controlled feeding and alcohol ingestion study. Journal of the National Cancer Institute 95(22):1722-1725.

Stacey Rucas 2004. Female Intersexual Behaviors Among the Tsimane of Bolivia. Doctoral Dissertation: UNM.

Ryan AS, and Elahi D. 1996. The effects of acute hyperglycemia and hyperinsulinemia on plasma leptin levels: its relationships with body fat, visceral adiposity, and age in women. The Journal of clinical endocrinology and metabolism 81(12):4433-4438.

Ryan AS, Berman DM, Nicklas BJ, Sinha M, Gingerich RL, Meneilly GS, Egan JM, and Elahi D. 2003. Plasma adiponectin and leptin levels, body composition, and glucose utilization in adult women with wide ranges of age and obesity. Diabetes care 26(8):2383-2388.

Saad MF, Riad-Gabriel MG, Khan A, Sharma A, Michael R, Jinagouda SD, Boyadjian R, and Steil GM. 1998. Diurnal and ultradian rhythmicity of plasma leptin: effects of gender and adiposity. The Journal of clinical endocrinology and metabolism 83(2):453-459.

Saad MF, Damani S, Gingerich RL, Riad-Gabriel MG, Khan A, Boyadjian R, Jinagouda SD, el-Tawil K, Rude RK, and Kamdar V. 1997. Sexual dimorphism in plasma leptin concentration. The Journal of clinical endocrinology and metabolism 82(2):579-584.

Santos-Alvarez J, Goberna R, Sanchez-Margalet V. 1999. Human Leptin Stimulates Proliferation

and Activation of Human Circulating Monocytes. Cellular Immunology. 194: 6-11.

Sarikonda KV, Watson RE, Opara OC, et al. 2009. Experimental animal models of hypertension. Journal of the American Society of Hypertension. 3 (3): 158-165.

Sartorio A, Agosti F, De Col A, Marinone PG, and Maffiuletti NA. 2004. Leptin levels do not change following acute exercise in healthy adults and in obese children. Journal of endocrinological investigation 27(10):992-993.

Sato M, Takeda N, Sarui H, Takami R, Takami K, Hayashi M, Sasaki A, Kawachi S, Yoshino K, and Yasuda K. 2001. Association between serum leptin concentrations and bone mineral density, and biochemical markers of bone turnover in adult men. J Clin Endocr Metab 86(11):5273-5276.

Sattar N, Greer IA, Pirwani I, et al. 1998. Leptin levels in pregnancy: marker for fat accumulation and mobilization? Acta Obstet Gynecol Scand. 77: 278-283.

Sharmanov A. 2000. Anemia testing manual for population based studies. Calverton: Macro International inc. pp. 38.

Schell LM 1995. Human plasticity: history and future research. In: Human variability and plasticity.

Scheper-Hughes N. 1987. Child survival: anthropological perspectives on the treatment and maltreatment of children. Boston, MA: Dordrecht.

Schmitz O, Fisker S, Orskov L, Hove KY, Nyholm B, and Moller N. 1997. Effects of hyperinsulinaemia and hypoglycaemia on circulating leptin levels in healthy lean males. Diabetes & metabolism 23(1):80-83.

Schoeller DA, Cella LK, Sinha MK, and Caro JF. 1997. Entrainment of the diurnal rhythm of plasma leptin to meal timing. Journal of Clinical Investigation 100(7):1882-1887.

Schrauwen P, van Marken Lichtenbelt WD, Westerterp KR, and Saris WH. 1997. Effect of diet composition on leptin concentration in lean subjects. Metabolism: clinical and experimental 46(4):420-424.

Schubring C, Englaro P, Sieblaer T, et al. 1998. Longitudinal Analysis of Maternal Serum Leptin Levels during Pregnancy, at Birth and up to six weeks after birth: relation to body mass index, skinfolds, sex steroids and umbilical cord blood leptin levels. Hormone Research. 50: 276-283.

Schubring C, Kiess W, Englaro P et al. 1997. Levels of leptin in maternal serum, amniotic fluid, and arterial and venous cord blood: relation to neonatal and placental weight. Journal of Clinical Endocrinology and Metabolism. 82(5): 1480-1483.

Schulz and Widmaier. 2006. Leptin Receptors. In: Leptin. Castracane VD and Henson MC eds. Singer, New York. 11-31.

Schwartz MW, Woods SC, Seeley RJ, et al. 2003. Is the energy homeostasis system inherently biased toward weight gain. Diabetes. 52(2): 232-8.

Schurgin S, Canavan B, Koutkia P, et al. 2004. Endocrine and Metabolic Effects of Physiologic r-metHuLeptin Administration during Acute Calorie Deprivation in Normal-Weight Women. The Journal of Clinical Endocrinology & Metabolism. 89 (11): 5402-5409.

Scriba D, Aprath-Husmann I, Blum WF, and Hauner H. 2000. Catecholamines suppress leptin release from in vitro differentiated subcutaneous human adipocytes in primary culture via beta1- and beta2-adrenergic receptors. European journal of endocrinology / European Federation of Endocrine Societies 143(3):439-445.

Seeman TE, and Crimmins E. 2001. Social environment effects on health and aging: integrating epidemiologic and demographic approaches and perspectives. Annals of the New York Academy of Sciences 954:88-117.

Segal KR, Landt M, and Klein S. 1996. Relationship between insulin sensitivity and plasma leptin concentration in lean and obese men. Diabetes 45(7):988-991.

Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, McDonald-Smith GP, Gao H, Hennessy L et al. 2013. Genomic responses in mouse models poorly mimic human inflammatory diseases. Proceedings of the National Academy of Sciences of the United States of America 110(9):3507-3512.

Shapses SA and Riedt CS. 2006. Bone, Body Weight, and Weight Reduction: What are the Concerns? Journal of Nutrition. 1453-1456.

Sharmonov A. 2000. Anemia Testing Manual for Population-based surveys. Calverton, MA: Macro International Inc.

Sharrock KC, Kuzawa CW, Leonard WR, Tanner S, Reyes-Garcia VE, Vadez V, Huanca T, and McDade TW. 2008. Developmental changes in the relationship between leptin and adiposity among Tsimane children and adolescents. American journal of human biology : the official journal of the Human Biology Council 20(4):392-398.

Shephard RJ. 1991. Body Composition in Biological Anthropology. Cambridge University Press: Cambridge.

Shih LY, Liou TH, Chao JC, Kau HN, Wu YJ, Shieh MJ, Yeh CY, and Han BC. 2006. Leptin, superoxide dismutase, and weight loss: initial leptin predicts weight loss. Obesity (Silver Spring) 14(12):2184-2192.

Sierra-Johnson J, Romero-Corral A, Somers VK, and Johnson BD. 2008. Last word on viewpoint: effect of altitude on leptin levels, does it go up or down? J Appl Physiol 105(5):1691.

Silha JV, Nyomba BL, Leslie WD, and Murphy LJ. 2007. Ethnicity, Insulin Resistance, and Inflammatory Adipokines in Women at High and Low Risk for Vascular Disease. Diabetes Care. 30: 286-291.

Sinha MK, Sturis J, Ohannesian J, Magosin S, Stephens T, Heiman ML, Polonsky KS, and Caro JF. 1996. Ultradian oscillations of leptin secretion in humans. Biochem Bioph Res Co 228(3):733-738.

Sinha MK, and Caro JF. 1998. Clinical aspects of leptin. Vitamins and hormones 54:1-30.

Simmons D and Breier BH. 2002. Fetal Overnutrition in Polynesian Pregnancies and in Gestational diabetes may lead to dysregulation of the adiposinsular axis in offspring. Diabetes Care. 25: 1539-1544.

Simsch C, Lormes W, Petersen KG, Baur S, Liu Y, Hackney AC, Lehmann M, and Steinacker JM. 2002. Training intensity influences leptin and thyroid hormones in highly trained rowers. International journal of sports medicine 23(6):422-427.

Siri WE. 1956. Gross composition of the body. in Advances in Biological and Medical Physics, vol. IV, edited by J.H. Lawrence and C.A. Tobias, Academic Press, Inc., New York.

Sivan E, Whittaker PG, Sinha D, et al. 1998. Leptin in human pregnancy: The relationship with gestational hormones. Am J Obstet Gynecol. 179: 1128-32.

Skurk T, Alberti-Huber C, Herder C, Hauner H. 2007. Relationship between adipocyte size and adipokine expression and secretion. The Journal of Clinical Endocrinology and Metabolism. 92(3): 1023-1033.

Soares MJ, Piers LS, O'Dea K, and Collier GR. 2000. Plasma leptin concentrations, basal metabolic rates and respiratory quotients in young and older adults. International journal of obesity and related metabolic disorders : journal of the International Association for

the Study of Obesity 24(12):1592-1599.

Sobhani I, Bado A, Vissuzaine C, Buyse M, Kermorgant S, Laigneau JP, Attoub S, Lehy T, Henin D, Mignon M and others. 2000. Leptin secretion and leptin receptor in the human stomach. Gut 47(2):178-183.

Somoza B, Guzman R, Cano V, et al. 2007. Induction of cardiac uncoupling protein-2 expression and adenosine 5'-monophosphate-activated protein kinase phosphorylation during early states of diet-induced obesity in mice. 148(3): 924-31.

Spiegel K, Leproult R, L'Hermite-Baleriaux M, Copinschi G, Penev PD, and Van Cauter E. 2004. Leptin levels are dependent on sleep duration: Relationships with sympathovagal balance, carbohydrate regulation, cortisol, and thyrotropin. J Clin Endocr Metab 89(11):5762-5771.

Spiegel K, Knutson K, Leproult R, Tasali E, and Van Cauter E. 2005. Sleep loss: a novel risk factor for insulin resistance and Type 2 diabetes. J Appl Physiol 99(5):2008-2019.

Staiger H, Tschritter O, Machann J, Thamer C, Fritsche A, Maerker E, Schick F, Haring HU, and Stumvoll M. 2003. Relationship of serum adiponectin and leptin concentrations with body fat distribution in humans. Obesity research 11(3):368-372.

Stanhope KL, Griffen SC, Bair BR, Swarbrick MM, Keim NL, and Havel PJ. 2008. Twenty-four-hour endocrine and metabolic profiles following consumption of high-fructose corn syrup-, sucrose-, fructose-, and glucose-sweetened beverages with meals. The American journal of clinical nutrition 87(5):1194-1203.

Stein AD, Wang M, Martorell R, Norris SA, Adair LS, Bas I, Sachdev HS, Bhargava SK, Fall CH, Gigante DP et al. . 2010. Growth patterns in early childhood and final attained stature: data from five birth cohorts from low- and middle-income countries. American journal of human biology : the official journal of the Human Biology Council 22(3):353-359.

Stini WA. 1972. Reduced Sexual Dimorphism in Upper Arm Muscle Circumference Associated with Protein-Deficient Diet in a South-American Population. American journal of physical anthropology 36(3):341-&.

Stoving RK, Vinten J, Handberg A, Ebbesen EN, Hangaard J, Hansen-Nord M, Kristiansen J, and Hagen C. 1998. Diurnal variation of the serum leptin concentration in patients with anorexia nervosa. Clinical endocrinology 48(6):761-768.

Strobel A, Issad T, Camoin L, et al. 1998. A leptin missense mutation associated with hypogonadism and morbid obesity. Nat Genet. 18(3): 213-5.

Strychar I, Lavoie ME, Messier L, Karelis AD, Doucet E, Prud'homme D, Fontaine J, and Rabasa-Lhoret R. 2009. Anthropometric, metabolic, psychosocial, and dietary characteristics of overweight/obese postmenopausal women with a history of weight cycling: a MONET (Montreal Ottawa New Emerging Team) study. Journal of the American Dietetic Association 109(4):718-724.

Sumner AE, Kushner H, Tulenko TN, Falkner B, and Marsh JB. 1997. The relationship in African-Americans of sex differences in insulin-mediated suppression of nonesterified fatty acids to sex differences in fasting triglyceride levels. Metabolism: clinical and experimental 46(4):400-405.

Supa AZ Gurven M, , and Kaplan H. 2007. Mortality experience of Tsimane Amerindians of Bolivia: regional variation and temporal trends. American journal of human biology : the official journal of the Human Biology Council 19(3):376-398.

Tamas P, Sulyok E, Szabo I, et al. 1998. Changes of Maternal Serum Leptin Levels during Pregnancy. Gynecol Obstet Invest. 46: 169-171.

Tamura T, Goldenberg RL, Johnston KE, et al. 1998. Serum Leptin Concentrations During Pregnancy and Their Relationship to Fetal Growth. Obstetrics and Gynecology. 389-95.

Tanaka M, Umezaki M, Natsuhara K, Yamauchi T, Inaoka T, Hongo T, Nagano M, Watanabe C, and Ohtsuka R. 2005. No difference in serum leptin concentrations between urban-dwelling Austronesians and Non-Austronesians in Papua New Guinea. American journal of human biology : the official journal of the Human Biology Council 17(6):696-703.

Tanner SN. 2005. A population in transition: health, culture change, and intestinal parasitism among the Tsimane' of lowland Bolivia PhD dissertation, The University of Michigan.

Taggart NR, Holliday RM, Billewicz WZ, Hytten FE, Thomson AM. 1967. Changes in skinfolds during pregnancy. British Journal of Nutrition. 21: 439-51.

Tartaglia LA, Dembski M, Weng X, et al. 1995. Identification and expression cloning of a leptin receptor, OB-R. Cell. 83 (7): 1263-71.

Teff KL, Elliott SS, Tschop M, Kieffer TJ, Rader D, Heiman M, Townsend RR, Keim NL, D'Alessio D, and Havel PJ. 2004. Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin, and increases triglycerides in women. The Journal of clinical endocrinology and metabolism 89(6):2963-2972.

Tennekoon KH, Wasalathanthri S, Jeevathayaparam S, Karunanayake EH. 2005. Serum leptin and lactational amenorrhea in well-nourished and undernourished lactating women. Fertility and Sterility. 83: 988-94.

Teppa RJ, Ness RB, Crombleholme WR, Roberts JM. 2000. Free leptin is increases in normal pregnancy and further increased in preeclampsia. Metabolism. 49(8): 1043-1048.

Thomas T and Burguera B. 2002. Is leptin the link between fat and bone mass? Journal of Bone and Mineral Research. 17(9): 1563-1569.

Thomas T, Burguera B, Melton LJ, Atkinson EJ, O'Fallon WM, Riggs BL, and Khosla S. 2001. Role of serum leptin, insulin, and estrogen levels as potential mediators of the relationship between fat mass and bone mineral density in men versus women. Bone 29(2):114-120.

Thong FS, McLean C, and Graham TE. 2000. Plasma leptin in female athletes: relationship with body fat, reproductive, nutritional, and endocrine factors. J Appl Physiol 88(6):2037-2044.

Torgerson JS, Carlsson B, Stenlof K, Carlsson LMS, Bringman E, Sjostrom L. 1999. A low serum leptin level at baseline and a large early decline in Leptin predicts a large 1-year weight reduction in energy-restricted obese humans. The Journal of Clinical Endocrinology and Metabolism. 84 (11): 4197-4203.

Togo M, Hashimoto Y, Futamura A, Tsukamoto K, Satoh H, Hara M, Watanabe T, Nakarai H, Nakahara K, and Kimura S. 2000. Relationship between the serum level of leptin and life-style habits in Japanese men. Hormone research 54(4):169-173.

Tomaszewski M, Charchar FJ, Przybycin M, Crawford L, Wallace AM, Gosek K, Lowe GD, Zukowska-Szczechowska E, Grzeszczak W, Sattar N et al. . 2003. Strikingly low circulating CRP concentrations in ultramarathon runners independent of markers of adiposity: how low can you go? Arteriosclerosis, thrombosis, and vascular biology 23(9):1640-1644.

Tommaselli GA, Pighetti M, Nasti A, et al. 2004. Serum leptin levels and uterine Doppler flow velocimetry at 20 weeks' gestation as markers for the development of pre-eclampsia. Gynecol Endocinol. 19:160-165.

Tong J, Fujimoto WY, Kahn SE, Weigle DS, McNeely MJ, Leonetti DL, Shofer JB, and Boyko EJ. 2005. Insulin, C-peptide, and leptin concentrations predict increased visceral adiposity at 5- and 10-year follow-ups in nondiabetic Japanese Americans. Diabetes 54(4):985-990.

Torjman MC, Zafeiridis A, Paolone AM, Wilkerson C, and Considine RV. 1999. Serum leptin during recovery following maximal incremental and prolonged exercise. International journal of sports medicine 20(7):444-450.

Trayhurn P, and Beattie JH. 2001. Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. The Proceedings of the Nutrition Society 60(3):329-339.

Tsao TH, Yang JB, Yang CB, Hsu CH, and Liou TL. 2007. The soluble leptin receptor of regular exercisers. International journal of sports medicine 28(9):732-735.

Tschop MH, Hui DY, Horvath TL. 2007. Diet-induced leptin resistance: the heart of the matter. Endocrinology. 148(3):921-3.

Tuominen JA, Ebeling P, Laquier FW, Heiman ML, Stephens T, and Koivisto VA. 1997. Serum leptin concentration and fuel homeostasis in healthy man. European journal of clinical investigation 27(3):206-211.

Ulijaszek SJ and Strickland SS. 1993. Nutritional Anthropology: Biological Perspectives. Smith-Gordon & Co Ltd.

Ulijaszek SJ. 1992. Human Energetic Methods in Biological Anthropology. Yearbook of Physical Anthropology. 35: 215-242.

Unal M, Unal DO, Baltaci AK, Mogulkoc R, and Kayserilioglu A. 2005. Investigation of serum leptin levels in professional male football players and healthy sedentary males. Neuro endocrinology letters 26(2):148-151.

Unkila-Kallio L, Andersson S, Koistinen HA, Karonen SL, Ylikorkala O, and Tiitinen A. 2001. Leptin during assisted reproductive cycles: the effect of ovarian stimulation and of very early pregnancy. Hum Reprod 16(4):657-662.

Usui C, Takahashi E, Gando Y, Sanada K, Oka J, Miyachi M, Tabata I, and Higuchi M. 2007. Relationship between blood adipocytokines and resting energy expenditure in young and elderly women. Journal of nutritional science and vitaminology. 53(6):529-535.

Utriainen T, Malmstrom R, Makimattila S, and Yki-Jarvinen H. 1996. Supraphysiological hyperinsulinemia increases plasma leptin concentrations after 4 h in normal subjects. Diabetes. 45(10):1364-1366.

Vadez V, Reyes-Garc V, Godoy RA, Apaza L, Byron E, Huanca T, Leonard WR, Perez E, and Wilkie D. 2004. Does Integration to the Market Threaten Agricultural Diversity? Panel and Cross-Sectional Data From a Horticultural-Foraging Society in the Bolivian Amazon.

Human Ecology. 32(5): 635-646.

Valeggia CR. 2007. Taking the Lab to the Field: Monitoring reproductive hormones in population-scale research. Population Development Review. 33(3): 525-542.

van Aggel-Leijssen DP, van Baak MA, Tenenbaum R, Campfield LA, and Saris WH. 1999. Regulation of average 24h human plasma leptin level; the influence of exercise and physiological changes in energy balance. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 23(2):151-158.

van Rossum CT, Hoebee B, van Baak MA, Mars M, Saris WH, and Seidell JC. 2003. Genetic variation in the leptin receptor gene, leptin, and weight gain in young Dutch adults. Obesity research 11(3):377-386.

Varvaigou A, Mantzoros CS, Beratis NG. 1999. Cord blood leptin concentrations in relation to intrauterine growth. Clinical Endocrinology. 50: 177-183.

Veniant MM, LeBel CP. 2003. Leptin: from animals to human. Curr Pharm Des. 9(10): 811-8.

Verdich C, Madsen JL, Toubro S, Buemann B, Holst JJ, and Astrup A. 2000. Effect of obesity and major weight reduction on gastric emptying. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 24(7):899-905.

Verhaeghe J, van Bree R, Van Herk E. 2006. Maternal body size and birth weight: can insulin or adipokines do better? Metabolism Clinical and Experimental. 55: 339-344.

Vettor R, De Pergola G, Pagano C, Englaro P, Laudadio E, Giorgino F, Blum WF, Giorgino R, and Federspil G. 1997. Gender differences in serum leptin in obese people: relationships with testosterone, body fat distribution and insulin sensitivity. European journal of clinical investigation 27(12):1016-1024.

Vidal H, Auboeuf D, De Vos P, Staels B, Riou JP, Auwerx J, and Laville M. 1996. The expression of ob gene is not acutely regulated by insulin and fasting in human abdominal subcutaneous adipose tissue. The Journal of clinical investigation 98(2):251-255.

Villanueva EC and Myers MG Jr. 2008. Leptin receptor signaling and the regulation of mammalian physiology. Int J Obes. 32 (Suppl 7): S8-12.

Vitoratos N, Chrystodoulacos G, Salamalekis E et al. 2002. Fetoplacental leptin levels and their relation to birth weight and insulin in gestational diabetic pregnant women. Journal of

Obstetrics and Gynaecology. 22(1): 29-33.

Vitoratos N, Salamalekis E, Kassanos D, et al. 2001. Maternal Plasma leptin levels and their relationship to insulin and glucose in gestational-onset diabetes. Gynecol Obstet Invest. 51:17-21.

Vitzthum VJ, Spielvogel H, Thornburg J. 2004. Interpopulational differences in progesterone levels during conception and implantation in humans. PNAS. 101 (6): 1443-1448.

Wabitsch M, Blum WF, Muche R, Braun M, Hube F, Rascher W, Heinze E, Teller W, and Hauner H. 1997. Contribution of androgens to the gender difference in leptin production in obese children and adolescents. The Journal of clinical investigation 100(4):808-813.

Wabitsch M, Jensen PB, Blum WF, Christoffersen CT, Englaro P, Heinze E, Rascher W, Teller W, Tornqvist H, and Hauner H. 1996. Insulin and cortisol promote leptin production in cultured human fat cells. Diabetes 45(10):1435-1438.

Wadden TA, Considine RV, Foster GD, Anderson DA, Sarwer DB, and Caro JS. 1998. Short- and long-term changes in serum leptin dieting obese women: effects of caloric restriction and weight loss. The Journal of clinical endocrinology and metabolism 83(1):214-218.

Wallace AM, McMahon AD, Packard CJ, et al. 2001. Plasma Leptin and the Risk of Cardiovascular Disease in the West of Scotland Coronary Prevention Study (WOSCOPS). Circulation. 104: 3052-3056.

Walker R, Gurven M, Hill K, Migliano A, Chagnon N, De Souza R, Djurovic G, Hames R, Hurtado AM, Kaplan H et al. . 2006. Growth rates and life histories in twenty-two small-scale societies. American journal of human biology : the official journal of the Human Biology Council 18(3):295-311.

Wang J and Deurenberg P. 1996. The validity of predicted body composition in Chinese adults from anthropometry and bioelectrical impedance in comparison with densitometry. British Journal of Nutrition. 175-182.

Wauters M, and Van Gaal L. 1999. Gender differences in leptin levels and physiology: a role for leptin in human reproduction. The journal of gender-specific medicine : JGSM : the official journal of the Partnership for Women's Health at Columbia 2(5):46-51.

Wei M, Stern MP, and Haffner SM. 1997. Serum leptin levels in Mexican Americans and non-Hispanic whites: association with body mass index and cigarette smoking. Annals of epidemiology 7(2):81-86.

Weigle DS, Duell PB, Connor WE, Steiner RA, Soules MR, and Kuijper JL. 1997. Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. The Journal of clinical endocrinology and metabolism 82(2):561-565.

Weiss LA, Barrett-Connor E, von Muhlen D, and Clark P. 2006. Leptin predicts BMD and bone resorption in older women but not older men: The Rancho Bernardo study. J Bone Miner Res 21(5):758-764.

Wellhoener P, Fruehwald-Schultes B, Kern W, Dantz D, Kerner W, Born J, Fehm HL, and Peters A. 2000. Glucose metabolism rather than insulin is a main determinant of leptin secretion in humans. The Journal of clinical endocrinology and metabolism 85(3):1267-1271.

Wells JCK. 2010. The evolutionary biology of human body fatness : thrift and control. Cambridge, UK ; New York: Cambridge University Press. xi, 382 p. p.

Welt CK, Chan JL, Bullen J, et al. 2004. Recombinant Human Leptin in women with hypothalamic amenorrhea. New England journal of medicine. 351: 987-997.

Weltman A, Pritzlaff CJ, Wideman L, Considine RV, Fryburg DA, Gutgesell ME, Hartman ML, and Veldhuis JD. 2000. Intensity of acute exercise does not affect serum leptin concentrations in young men. Medicine and science in sports and exercise 32(9):1556-1561.

Westererp-Plantenga MS, Saris WHM, Huckshorn CJ, Campfield LA. 2001. Effects of Weekly administration of pegylated recombinant human OB protein on appetite profile and energy metabolism in obese men. American Journal of Clinical Nutrition. 74: 426-34.

Widdowson PS, Upton R, Buckingham R, Arch J, Williams G. 1997. Inhibition of food response to intracerebroventricular injection of leptin is attenuated in rats with diet-induced obesity. Diabetes. 46(11):1782-5.

Williams F. 1991. Reasoning with Statistics: How to read quantitative research, 4th edition. Fort Worth: Harcourt College Publishers.

Williams MA, Havel PJ, Schwartz MW, et al. 1999. Preeclampsia disrupts the normal relationship between serum leptin concentrations and adiposity in pregnant women. Pediatric and perinatal epidemiology. 13: 190-204.

Williams SM, Haines JL, Moore JH. 2004. The use of animal models in the study of complex disease: all else is never equal or why do so many human studies fail to replicate animal findings. BioEssays. 26: 170-179.

Wing RR, Sinha MK, Considine RV, Lang W, and Caro JF. 1996. Relationship between weight loss maintenance and changes in serum leptin levels. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 28(12):698-703.Wolfe BE, Jimerson DC, Orlova C, and Mantzoros CS. 2004. Effect of dieting on plasma leptin, soluble leptin receptor, adiponectin and resistin levels in healthy volunteers. Clinical endocrinology 61(3):332-338.

Winking J. 2005. Fathering among the Tsimane of Bolivia: A Test of the Proposed Goals of Paternal Care. Doctoral Dissertation: UNM.

Winking J, Kaplan H, Gurven M, and Rucas S. 2007. Why do men marry and why do they stray? Proceedings Biological sciences / The Royal Society 274(1618):1643-1649.

Wong SL, Depaoli AM, Lee JH, Mantzoros CS. 2004. Leptin Hormonal Kinetics in the Fed State: Effects of Adiposity, Age, and Gender on Endogenous Leptin Production and Clearance Rates. 89 (6): 2672-2677.

Wood JW. 1994. Dynamics of Human Reproduction: Biology, Biometry, Demography. Aldine de Gruyter: New York.

Worthman CM. 2003. Energetics, Sociality, and Human Reproduction: Life History Theory in Real Life. In: Offpsring: Human Fertility Behavior in Biodemographic Perspective.

Worthman CM and Stallings JF. 1997. Hormone measures in finger-prick blood spot samples: New field methods for reproductive endocrinology. American Journal of Physical Anthropology104: 1–21

Worthman C M and Stallings JF.1994. Measurement of gonadotropins in dried spots. Clinical Chemistry. 40: 448–453.

www.tsimane.org- Tsimane' Amazonian Panel Study.

Yang M-J. 2005. Interrelationships of Maternal Serum Leptin, Body Mass Index, and Gestational Age. J Chin Med Assoc. 68(10): 452-457.

Yamauchi M, Sugimoto T, Yamaguchi T, Nakaoka D, Kanzawa M, Yano S, Ozuru R, Sugishita T, and Chihara K. 2001. Plasma leptin concentrations are associated with bone mineral

density and the presence of vertebral fractures in postmenopausal women. Clin Endocrinol 55(3):341-347.

Yannakoulia M, Yiannakouris N, Bluher S, Matalas AL, Klimis-Zacas D, and Mantzoros CS. 2003. Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. The Journal of clinical endocrinology and metabolism 88(4):1730-1736.

Yildiz L, Avci B, Ingec M. 2002. Umbilical Cord and Maternal Blood Leptin Concentrations in Intrauterine Growth Retardation. Clin Chem Lab Med. 40(11): 1114-1117.

Yoshinari M, Wakisaka M, and Fujishima M. 1998. Serum leptin levels in smokers with type 2 diabetes. Diabetes care 21(4):516-517.

Yura S, Sagawa N, Mise H, et al. 1998. A positive umbilical venous-arterial difference of leptin level and its rapid decline after birth. Am J Obstet Gynecol. 178:926-30.

Zafeiridis A, Smilios I, Considine RV, and Tokmakidis SP. 2003. Serum leptin responses after acute resistance exercise protocols. J Appl Physiol 94(2):591-597.

Zarkesh-Esfahani H, Pockley G, Metcalfe RA, et al. 2001. High-Dose leptin activates human leukocytes via receptor expression on monocytes. The Journal of immunology. 167: 4593-4599.

Zelissen PM, Stenlof K, Lean ME, et al. 2005. Effect of three treatment schedules of recombinant methionyl human leptin on body weight in obese adults: a randomized, placebo-controlled trial. Diabetes Obes Metab. 7(6): 755-61.

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. 1994. Positional cloning of the mouse obese gene and its human homologue. Nature. 372 (6505): 425–32.

Zoladz JA, Konturek SJ, Duda K, Majerczak J, Sliwowski Z, Grandys M, and Bielanski W. 2005. Effect of moderate incremental exercise, performed in fed and fasted state on cardio-respiratory variables and leptin and ghrelin concentrations in young healthy men. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society 56(1):63-85.

Zumbach MS, Boehme MW, Wahl P, Stremmel W, Ziegler R, and Nawroth PP. 1997. Tumor necrosis factor increases serum leptin levels in humans. The Journal of clinical endocrinology and metabolism 82(12):4080-4082.