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Original Research Article

Developmental Changes in the Relationship Between Leptin and Adiposity Among Tsimané Children and Adolescents

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ABSTRACT Leptin is thought to signal energy stores, thus helping the body balance energy intake and expenditure. However, the strong relationship between leptin and adiposity in populations with adequate nutrition or common obesity is not universal across ecologic contexts, and leptin often correlates only weakly, or not at all, with adiposity in populations of lean or marginally-nourished males. To clarify whether the relationship between adiposity and leptin changes during development, this study examines leptin and body fat among children and adolescents of lowland Bolivia. Anthropometric measures of body composition and dried blood spot samples were collected from 487 Tsimane' ranging from 2 to 15 years of age. Leptin was assayed using an enzyme immunoassay protocol validated for use with blood spot samples. In this population, leptin concentrations were among the lowest reported in a human population (mean ± SD: 1.26 ± 0.5 and 0.57 ± 0.3 in females and males). In addition, the relationship between leptin and adiposity follows distinct developmental trajectories in males and females. In males, leptin is weakly correlated with most measures of body composition at all ages investigated. However, in females, the level of body fat and the strength of the correlation between body fat and leptin (a measure of its strength as a signal of energy stores) both increase markedly with age. These findings suggest a more important role of leptin as a signal of energy stores among females as they approach reproductive maturity, while raising questions about the function of this hormone in lean males. Am. J. Hum. Biol. 20:392–398, 2008.

The dynamic between energy intake and expenditure strongly influences key biological processes such as somatic maintenance, growth, and reproduction (Leonard and Ulijaszek, 2002). As such, the body's ability to monitor its energy status is critical to an organism's survival and reproductive success. Leptin, a hormone produced in fat cells, has been proposed as a signal of total energy reserves, thus helping organisms maintain energetic homeostasis. Leptin helps the body to maintain energy balance both by influencing hypothalamic nuclei in the central nervous system that regulate energy expenditure and intake, and through direct effects on peripheral functions and tissues like the reproductive organs, immune system, and skeletal growth (Bjorbaek and Kahn, 2004; Margetic et al., 2002). The broad functions of the hormone are demonstrated in *ob*/*ob* knock-out mice that are incapable of producing leptin and in *db*/*db* knockout mice that lack leptin receptors. *Ob*/*ob* and *db*/*db* mice both experience increased appetite, decreased expenditure on functions like growth, immunity and reproduction, and excessive body fat gain (Bray and York, 1979; Ducy et al., 2000; Howard et al., 1999).

Leptin has largely been studied in relatively over-nourished Western clinical populations which are characterized by frequent overweight and development of the metabolic syndrome (Trayhurn, 2001). Strong correlations between measures of body fat and circulating leptin concentration are consistently found in these settings (Jequier, 2002). Thus, leptin is widely assumed to be an accurate indicator of available energy stores to the CNS in both males and females. Although the correlation between fat and leptin is widely documented in Western clinical settings, these findings are not universal. Studies in lean subsistence populations, although few in number, have shown that the relationship between leptin and adiposity may vary quite markedly between the sexes and across ecologic contexts. Among the few marginally-nourished populations that have been studied, leptin is often poorly associated, or not associated at all, with adiposity in males. The lack of association between leptin and fat stores among lean males raises questions about the hormone's ability to accurately reflect energy stores, thus casting doubt on its presumed function in managing energy balance in these settings (Bribiescas, 2001; Kuzawa et al., 2007).

Studies of leptin and body composition in lean populations have thus far been limited to adults or to samples of children and adolescents that represent a relatively narrow age range. Consequently, developmental changes in the relationship between adiposity and leptin have not been explored. To clarify the role of leptin as a signal of energy stores during development, this study examines

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leptin concentrations and their anthropometric correlates in a sample of Tsimané children and adolescents, ages 2–15 years, from lowland Bolivia. Tsimané children have a high burden of infectious disease that contributes to undernutrition and high levels of growth stunting (Foster et al., 2005; McDade et al., 2005, 2007; Tanner, 2005). The objectives of this study are as follows: (1) to document age changes in body fat distribution and leptin concentrations among Tsimané children and adolescents; (2) to evaluate the nature of the relationship between leptin and multiple measures of body composition or adiposity; and (3) to analyze changes in the relationship between leptin and adiposity in different age groups to help clarify the developmental basis of sex differences in leptin physiology.

MATERIALS AND METHODS

The Tsimané

The Tsimané are a population of approximately 8,000 living in the lowland rainforests of the Amazon basin, in the Department of Beni in Bolivia. They inhabit villages of 2–70 households and subsist primarily through swidden farming, supplemented to varying degrees by hunting and gathering and emerging opportunities for wage labor in logging camps or cattle ranches (Godoy et al., 2005). Their environment is characterized by considerable seasonal fluctuation, with annual cycles of heavy rains and drought. Historically, the Tsimané have remained relatively isolated, but during the latter part of the twentieth century greater contact with outsiders has led to increasing integration into the surrounding market economy. At the time of this study, only half of the villages were accessible year round by road, and electricity and running water were not available to any household.

Data collection

Data were collected as part of the ongoing Tsimané Amazonian Panel Study investigating the effects of cultural and economic transitions on Tsimané well-being. Thirteen communities were selected that vary in distance from the town of San Borja, the regional commercial center (population ~16,000). The data presented here were collected during the baseline survey, conducted in May through July of 2002.

An attempt was made to enroll all residents between the ages of 2 and 15 years into the study. Census information for this population is not available, so we cannot formally evaluate the proportion included in our sample. Anthropometric measures and blood spot samples were collected in a single day, and virtually everyone who was present that day was included in the sample. However, village residents who happened to be absent were not included. Complete data and dried blood spot samples were collected from a sample of 487 children and adolescents (261 males; 226 females). Anthropometrics were measured using standard techniques (Lohman et al., 1998). Standing height (cm), body weight (kg), four skinfolds (mm), and mid-upper arm circumference (MUAC; cm) were measured in light clothing and without shoes. Height was measured with a portable stadiometer to the nearest millimeter. Weight was measured using a standing scale to the nearest 0.2 kg. Triceps, biceps, subscapular, and suprailiac skinfold thicknesses were measured to the nearest 0.5 mm using Lange calipers. MUAC was measured using plastic tape measures to the nearest millimeter.

From the raw anthropometric data, a series of derived measures were calculated, including: (1) the body mass index (BMI; kg/m^2), (2) arm muscle area (AMA; cm^2), (3) percent body fatness, (4) fat mass (FM; kg), and (5) fat-free mass (FFM; kg). The BMI was calculated as [weight (kg)]/[height (m)]^2. AMA was calculated from the MUAC and tricep skinfold measures as follows:

$$\mathrm{AMA} = (\mathrm{MUAC} - \mathrm{Tr}[\pi])^2/(4\pi) \quad (\text{Frisancho, 1990}).$$

Percent body fatness was estimated from the sum of the triceps and subscapular skinfolds using the equations of Slaughter et al. (1988). From these estimates of body fatness, FM and FFM were calculated as FM = (pctfat) (weight)/100 and FFM = Weight − FM.

There are difficulties in using skinfold measures to estimate percent fatness in children, including the fact that predictive equations are often based on narrow age ranges with considerable variation in which skinfolds are used across different studies. The Slaughter et al. (1988) equations were used here because they were based on a wide range of ages. Moreover, we felt that it was most appropriate to use a single set of reference equations for all children, rather than mixing equations based on different sets of skinfolds for children of different ages. Because of the limitations associated with estimating percent fatness, and the fact that these equations have not been validated for use specifically in this population, the correlates of leptin levels are examined using both the raw skinfold measures and the transformed fatness estimates. Height-for-age, weight-for-age, and weight-for-height were standardized as *z*-scores relative to the US National Center for Health Statistics (NCHS) standards (Hamill et al., 1979) using the ANTHRO software (Sullivan and Gorstein, 1990).

Samples of whole blood were collected on filter paper and dried for later analysis of leptin. Each participant's finger was cleaned using alcohol wipes and punctured using a sterile, disposable microlancet, and free-flowing capillary blood was then applied to standardized filter paper (Whatman #903) (McDade et al., 2007). The blood samples were dried overnight and then transported to San Borja, where they were refrigerated before shipment to the Laboratory for Human Biology Research at Northwestern University. Samples were stored frozen at −30°C until analysis for leptin.

Prior analyses have shown that leptin concentrations degrade when dried blood spot samples are exposed to elevated temperatures (Miller et al., 2006). All samples were transported to San Borja and refrigerated in less than 48 h, with the exception of a small number of samples (n = 41) that were exposed to an extra day at tropical temperatures. Leptin concentrations in these samples are significantly lower (oneway ANOVA F = 5.49, P < 0.01), likely due to sample degradation. We have adjusted for this effect in all the analyses presented here. In addition, we conducted parallel analyses excluding these samples to confirm that their inclusion did not alter our results or conclusions.

The study protocol was approved by the Northwestern University Institutional Review Board for research involving human subjects. The Tsimané Grand Council also

TABLE 1. Measures of nutritional status and leptin concentrations by age and sex[a]

Age (years)	*n*	Stature (HAZ)	WAZ	WHZ	BMI (kg/m^2)	Adiposity (% fat)	Bicep (cm)	Tricep (cm)	Subscapular (cm)	Suprailiac (cm)	Leptin (ng/ml)
Females											
All	226	−1.7 (1.1)	−0.9 (0.9)	0.4 (0.9)	17.3 (2.5)	15.8 (5.2)	4.4 (1.7)	8.9 (3.3)	8.1 (3.8)	8.8 (4.3)	1.26 (1.50)
2–3	35	−1.8 (0.9)	−0.8 (0.8)	0.4 (0.9)	16.9 (1.5)	13.6 (3.1)	5.1 (1.7)	8.0 (2.4)	6.4 (1.4)	7.5 (2.9)	0.78 (0.41)
4–6	58	−1.8 (1.3)	−0.9 (1.0)	0.3 (0.8)	16.0 (1.3)	13.4 (3.0)	4.0 (1.3)	7.7 (2.0)	6.3 (1.6)	6.6 (2.5)	0.65 (0.32)
7–9	49	−1.7 (1.4)	−1.1 (1.0)	0.3 (1.2)	16.0 (1.8)	13.7 (2.8)	3.4 (1.1)	7.6 (2.1)	6.6 (1.5)	7.0 (2.6)	0.77 (0.40)
10–12	50	−1.5 (0.8)	−0.7 (0.7)	NA	18.1 (2.0)	17.3 (4.7)	4.4 (1.5)	9.4 (3.4)	9.1 (3.2)	10.7 (4.2)	1.28 (0.97)
13–15	34	−1.7 (0.6)	−0.7 (0.9)	NA	20.9 (2.8)	22.7 (5.9)	5.8 (2.1)	12.8 (3.7)	13.4 (5.3)	13.4 (5.3)	3.51 (2.62)
Males											
All	261	−1.7 (1.2)	−0.9 (1.0)	0.4 (0.9)	17.1 (1.7)	10.8 (2.7)	3.6 (1.2)	6.9 (2.2)	6.0 (1.4)	6.1 (2.5)	0.57 (0.32)
2–3	34	−1.9 (1.3)	−1.0 (1.0)	0.2 (0.9)	17.0 (1.7)	12.0 (2.6)	4.4 (1.7)	7.8 (1.9)	5.9 (1.3)	6.3 (2.1)	0.64 (0.35)
4–6	72	−1.6 (1.5)	−0.7 (1.2)	0.5 (1.0)	16.5 (1.4)	10.8 (2.1)	3.7 (1.3)	6.9 (1.8)	5.5 (1.1)	5.9 (2.6)	0.59 (0.26)
7–9	73	−1.8 (1.3)	−0.9 (0.9)	0.5 (0.9)	16.5 (1.4)	10.7 (2.7)	3.5 (1.1)	6.3 (2.1)	5.9 (1.4)	5.9 (2.6)	0.53 (0.31)
10–12	57	−1.6 (1.0)	−1.0 (0.7)	0.4 (0.6)	17.4 (1.3)	10.4 (2.7)	3.2 (0.7)	6.8 (2.0)	6.3 (1.6)	6.1 (2.6)	0.58 (0.39)
13–15	25	−1.3 (0.6)	−0.7 (0.7)	NA	19.8 (1.5)	10.5 (3.7)	3.6 (0.7)	7.9 (3.8)	7.0 (1.3)	7.1 (2.2)	0.45 (0.21)

[a]Mean (SD).

approved the study, and parental consent as well as child/adolescent assent was obtained prior to enrollment.

Laboratory analysis of leptin

Leptin concentrations were determined using a highly sensitive sandwich enzyme immunoassay protocol developed specifically for use with dried blood spot samples (Miller et al., 2006). Dried blood spot disks were eluted overnight in assay buffer. Blood spot eluate was added to antibody-coated microplate wells and incubated with detection antibody, streptavidin-horseradish peroxidase conjugate, and then chromogenic substrate. Color change was read at 450 nm. The lower detection limit for this protocol is 0.15 ng/ml, and the correlation between leptin concentrations in matched blood spot and plasma samples is high (Pearson $R = 0.976$). Day-to-day variation across assays was monitored using three control values that were included in every assay. The inter-assay percent coefficients of variation were 11%, 8%, and 9% for the low, medium, and high controls, respectively.

Statistical analyses

Complete leptin concentration and anthropometric data were available for 487 individuals. Blood spot leptin results were transformed into plasma equivalent concentrations based on analysis of 63 matched plasma and blood spot samples, in which the correlation between plasma and blood spot results was determined to be linear and high (plasma leptin = 2.1171 × blood spot leptin − 0.0455; Miller et al., 2006). Transformed values are presented in order to facilitate comparisons with prior leptin research, although such comparisons should be made with caution due to differences in methods across labs and protocols.

Statistical analyses were performed using Stata version 8.2 (Stata Corp., 2005, College Station, TX). Leptin concentrations and measures of adiposity were log-transformed to normalize the distributions prior to analysis. Partial correlation coefficients were calculated to describe the relationships between leptin and measures of body composition and nutritional status adjusting for potential confounding factors. Pearson's correlation coefficients were calculated across age and sex categories to evaluate changes in the relationship between leptin and measures of nutritional status.

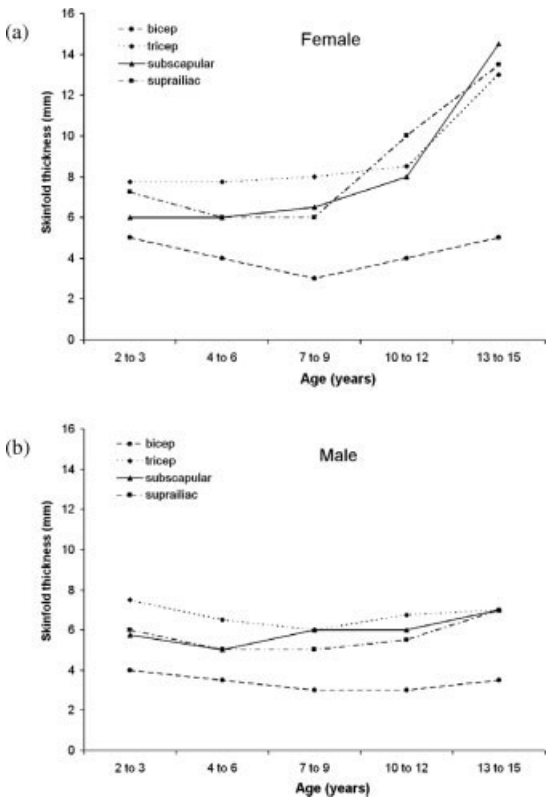

Fig. 1. **a,b**) Median skinfold thicknesses by age group in Tsimané females and males.

RESULTS

Table 1 presents the mean concentrations of leptin and anthropometric measures of growth and adiposity stratified by sex and age. As documented previously (Foster et al., 2005), the Tsimané show a high level of linear growth stunting in males and females. The population is lean, with low average BMI as compared to most US or European populations.

Figure 1a,b show median values for four skinfolds by age in females and males. In females, skinfold thicknesses stay relatively constant from ages 2 to 11. All skinfolds

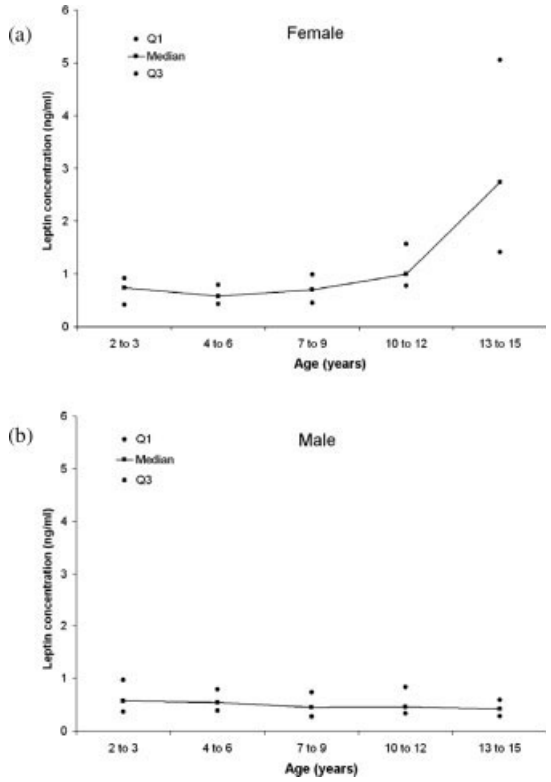

Fig. 2. **a,b**) Leptin concentrations by age group in Tsimané females and males.

other than biceps increase considerably in the oldest female age group. In males, skinfold thicknesses are substantially lower than in females and stay nearly constant across all age groups.

Figure 2a,b plot the median and interquartile range for leptin concentrations by age group in females and males. Leptin levels are very low overall and are higher in females than males throughout the age range of the sample. In females, leptin concentrations increase slightly from ages 2 to 12 and then increase dramatically in the oldest age group, 13–15. In males, leptin concentrations are consistently low across all age groups.

Table 2 reports the partial correlations between leptin and anthropometric measures of body composition (both logarithmically transformed) adjusting for age, sex and time that dried blood spots spent at tropical temperatures. These analyses show that the relationship between measures of body composition and leptin follow distinct developmental trajectories in males and females. In females, most correlations strengthen with age, suggesting that leptin becomes increasingly coupled with energy status. In males, in contrast, correlations are low and do not increase with age, suggesting that leptin may be a poor signal of energy status in males. There are few significant associations between leptin and any anthropometric measure in males of any age.

Figure 3a,b plot the partial correlations for the four skinfolds listed in Table 2 by age group in females and males, illustrating the much stronger relationship between adiposity and leptin in females, and the marked sex differences in the pattern of age changes in these relationships.

DISCUSSION

Consistent with their generally poor nutritional status and low adiposity, leptin concentrations among the Tsimané children and adolescents studied here are lower than reported in prior studies of children and adolescents. In females, skinfold thicknesses and leptin concentrations rise in parallel with age, while in males, there is minimal variation in skinfold thicknesses and leptin across the full age range of study participants, spanning 2–15 years of age. The strength of the relationship between leptin and adiposity, reflecting the degree to which leptin is coupled with energy stores, generally increases with age in females as they approach late childhood and adolescence. In males, in contrast, leptin is a poor correlate of adiposity across all ages. These findings provide insights into the development of sex differences in leptin biology previously documented in lean adults, and raise questions about the function of the hormone as a signal of energy stores in males.

As expected, skinfold thicknesses were greater in females than in males throughout the age distribution. The increase in skinfold thickness with age in females was expected as fat deposition is often rapid in females with the onset of puberty (Tanner, 1990). The concurrent rise in leptin with increasing adiposity was also expected. Similarly, the slight decrease in male skinfold thickness with age is consistent with expectations that lean mass will increase and FM will decrease in pubertal males (Tanner, 1990). However, many studies in better-nourished clinical samples report an increase of leptin concentrations in males into early puberty, parallel to that in females, before dropping off in late puberty (Ahmed et al., 1999; Clayton et al., 1997; Kratzsch et al., 2002). Although pubertal maturational status was not available for this sample, this pattern of change in leptin concentration appears not to be present among Tsimané males, perhaps reflecting differences in body composition or leptin physiology.

To our knowledge, this study is the first to present evidence for changes in the relationship between adiposity and leptin during the period of growth and development. These changes followed distinct trajectories in males and females. Leptin was generally more tightly coupled with body composition and adiposity measures among females in later childhood and adolescence, while these same correlations were rarely significant and did not differ consistently across age groups in males. Although there is no precedent for this finding, it is consistent with other studies of leptin in marginally nourished adults. Among the Aché of Paraguay, the predicted relationship between leptin and adiposity exists among adult females, but adult males display no correlation between leptin and adiposity (Bribiescas, 2001). When compared with US samples, Aché men and women have leptin levels similar to male marathon runners and female patients with anorexia nervosa, despite having higher levels of adiposity (Bribiescas, 2005; Bribiescas and Hickey, 2006). A study of lean Filipino adolescents found that leptin was strongly predicted by triceps and subscapular skinfolds in females but only by triceps skinfold in males, suggesting a weaker relationship between leptin and adiposity in males (Kuzawa et al., 2007). Additionally, leptin concentrations

TABLE 2. Partial correlation between leptin and anthropometric measures of nutritional status[a]

	Female					Male				
Age (years)	2–3	4–6	7–9	10–12	13–15	2–3	4–6	7–9	10–12	13–15
BMI	0.333	0.166	0.214	0.479***	0.454**	−0.279	0.010	0.225	0.173	0.315
Percent fat	0.329	0.376**	0.335*	0.648***	0.580***	−0.053	0.300*	0.217	0.242	0.124
Fat mass	0.327	0.450***	0.429**	0.676***	0.586***	−0.227	0.325**	0.230	0.195	0.0178
Biceps	0.461*	0.175	0.107	0.460**	0.562**	−0.167	0.012	0.161	0.110	−0.070
Triceps	0.468**	0.354**	0.225	0.571***	0.619***	−0.088	0.307**	0.171	0.322*	0.139
Subscapular	0.268	0.314*	0.353*	0.610***	0.534**	−0.028	0.176	0.228	0.112	0.134
Suprailiac	0.234	0.035	−0.026	0.477***	0.503**	−0.108	−0.071	0.098	−0.113	0.463*

[a]Adjusted for age, sex, and time dried blood spots spent at tropical temperatures.
*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.

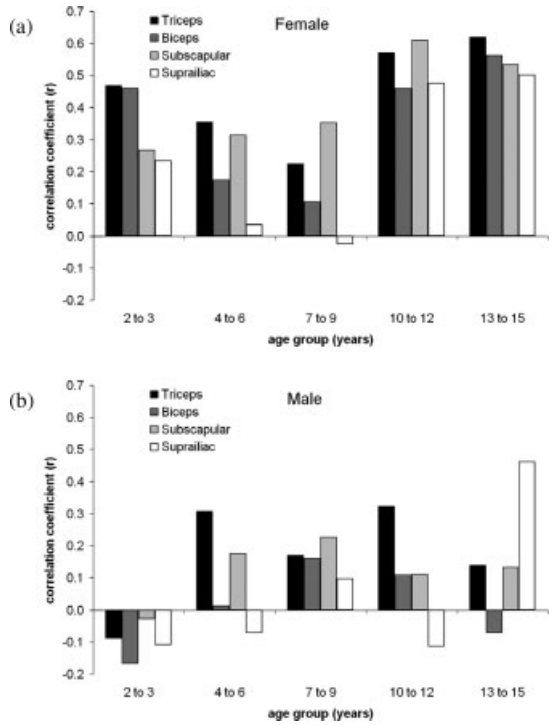

Fig. 3. **a,b**) Partial correlation coefficients relating plasma leptin to skinfold thickness by age group in Tsimané females and males (data from Table 2).

changed little across most of the skinfold distribution in leaner males, and the correlation with leptin was greatly reduced when the fattest 10% of the sample were excluded. The relationship between leptin and adiposity was also absent among malnourished Indian children living in slum conditions (Freeman et al., 2002) and weak among rural male Gambian children (Moore et al., 2002). These findings in humans are consistent with work on lean primates. While heavier, captive baboons show an association between body weight and leptin, leptin levels are lower and unrelated to body weight in wild baboons (Banks et al., 2001). Because obesity was likely rare during most of human evolution, it has been argued that leptin evolved to signal transitions between states of energy sufficiency and insufficiency, and may serve as a more sensitive measure of changes in weight (Prentice et al., 2002). Future analyses should incorporate longitudinal measures to evaluate this possibility.

The age-related increase in the correlations between leptin and adiposity measures in females indicate that, not only does body fatness increase as females approach adolescence, but the value of leptin as a signal of energy reserves also strengthens in parallel. This pattern hints at a more important role for leptin as energy signal among females as they approach reproductive maturity, and could be related to the energy requirements of sustaining pregnancy and lactation. This interpretation is supported by recent studies showing that leptin plays a permissive role in initiating puberty in females (Apter, 2003; Cervero et al., 2006; Kiess et al., 1998) and that low leptin concentrations prior to puberty predict subsequent gains in adiposity among girls during puberty (Ahmed et al., 1999). Together, these findings suggest that leptin could play a central role in allowing females to calibrate reproductive physiology to energy availability (Ellison, 1990), including the onset of reproductive maturation. In contrast, the weak correlations between adiposity measures and leptin in this and other lean populations of males suggest that leptin may have less importance as a signal of energy status in adult males (Bribiescas, 2001, Kuzawa et al., 2007). In the context of life history strategy (Stearns, 1992), the low energy requirements of sperm production could reduce the need to couple male maturation or reproduction with energy stores, with testosterone playing a more central role as regulator of male reproductive allocations (Bribiescas, 2001; Ellison, 2001).

Although the origins of population variation in leptin physiology remain to be clarified, several possibilities must be considered. In this study, we do not consider the influences of diet, activity level, disease status, or genetics, all of which have been shown to affect leptin concentrations. Variation in leptin and leptin receptor alleles exists across human populations and might factor into leptin physiology in the Tsimané. Although both the Aché and Tsimané are indigenous South American populations, the finding of similar relationships in Filipino male adolescents (Kuzawa et al., 2007) and Gambian male children (Moore et al., 2004) shows that these relationships are not specific to a particular subset of populations with shared genetic heritage. Further studies of leptin signaling in populations representing a range of body composition and nutritional ecologies will help clarify whether this pattern of sex differences in leptin physiology is more widespread.

The low leptin levels found among the Tsimane could also trace to developmental responses to early environments

(Gluckman and Hanson, 2006; Symonds et al., 2005), as an example of the widely-documented capacity for early nutrition to influence later metabolism and life history (Kuzawa, 2005). Restricting the protein intake of rat dams during pregnancy, lactation or both, reduces leptin levels in adult offspring (Zambrano et al., 2006), while rats exposed to protein restriction during fetal life show evidence for resistance to the effects of leptin on metabolism as adults (Krechowec et al., 2006). In human observational research, birth weight has been found to relate both positively and negatively with later leptin levels adjusted for adiposity (Jaquet et al., 2001; Phillips et al., 1999). Similarly, individuals born premature and randomized to receive banked breast milk had lower leptin levels per unit fat as adolescents than individuals randomized to receive formula, suggesting that postnatal nutrition and mode of infant feeding may also have lasting effects on later leptin physiology (Singhal et al., 2002). It is possible that the marginal nutritional status of the Tsimané, likely leading to undernutrition during the prenatal period, and high rates of breastfeeding, could contribute to their low levels of leptin relative to other populations. The contribution of developmental processes to population variation in leptin physiology warrants future research attention.

In summary, our findings contribute to the growing literature documenting leptin concentrations and physiology in lean, nonclinical samples. The weak relationship between leptin and adiposity in Tsimané males, along with similar findings in the Aché, Filipinos, and Gambians, challenges current assumptions of the function of leptin in energy signaling. In this sample, sex differences in the relationship between adiposity and leptin show a clear developmental trajectory, with the fat-leptin correlation weak across all ages in males, but strengthening markedly in females as they approach puberty. These findings hint at a more important role for leptin as a signal of energetic status in females, perhaps including a greater influence of this system on the timing of maturational events and reproductive strategy. Finally, we speculate that human population differences in the biology of leptin and its relationship with body composition may trace to organizational effects of early nutritional experiences in utero or during the early postnatal period.

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