

Protein content of diets dictates the daily energy intake of a free-ranging primate

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An important goal in nutritional ecology is to understand what governs the diet selection of free-living animals. Relevant information is however scarce because of the considerable challenges of collecting and interpreting such data. Here we use recent advances in nutritional theory to analyze data on food selection and nutrient intake by wild spider monkeys (*Ateles chamek*). We show that hypotheses traditionally used to explain vertebrate diet selection, such as energy or protein maximization, or avoidance of plant secondary metabolites, cannot explain the observed pattern of nutrient intake. Instead, spider monkeys maintained a stable daily protein intake but allowed total energy intake to vary as a function of the composition of available food items. A similar “protein-leverage effect” has been reported in humans for whom it appears to play a role in the development of obesity. *Key words:* *Ateles chamek*, nutrient balancing, nutritional ecology, primates, protein. [*Behav Ecol*]

Interactions between functional traits and ecological outcomes are to a large degree mediated by the regulatory mechanisms concerned with nutrient gain (Raubenheimer, Simpson and Mayntz, 2008). Understanding how an animal regulates its nutrient intake, which nutritional strategies it adheres to, and how these affect its fitness are therefore important goals in nutritional ecology. There are 4 major schools of thought in nutritional ecology, each of which proposes that diet selection subserves a different primary nutritional goal: 1) the maximization of energy intake (Schoener 1971); 2) the maximization of nitrogen intake (Mattson 1980; White 1993); 3) the avoidance of toxins (Freeland and Janzen 1974; Dearing et al. 2005); and 4) the acquisition of a nutritionally balanced diet (Raubenheimer and Simpson 2004; Robbins et al. 2007). Quantifying the relative influence of these different nutritional goals is challenging, especially in studies of wild animals. Research of this nature requires detailed data on feeding behavior from individual animals over continuous periods, relevant analyses of all foods consumed, and an analytical framework for dealing with the complex, multivariate nature of nutritional data.

Here, we apply recent advances in nutritional theory to a uniquely detailed field data set and demonstrate the nutritional goals of Peruvian spider monkeys (*Ateles chamek* Humboldt 1812, subfamily Atelinae) inhabiting a semideciduous forest in lowland Bolivia.

We observed the feeding behavior of focal animals (FAs) continuously from dawn to dusk, collected samples of their food, and analyzed the nutritional composition and the pres-

ence of certain plant secondary metabolites (PSMs). The macronutrient content of diets (we include carbohydrates, lipids and protein in the term “macronutrients”) differed widely during the 9-month study period, and the relative abundance of different food types varied markedly between seasons. These sources of variation allowed us to address the extent to which energy, protein, and nonnutrient chemicals determined patterns of intake. We analyzed the data using the Geometric Framework, a multidimensional approach designed to assess the relative priorities assigned by animals to different food components (Simpson and Raubenheimer 1993) (Figure 1a). Our results show that these frugivorous monkeys maintain protein intake relatively constant but allow fat and carbohydrate intake (and thus total energy intake) to vary as a function of the nutritional composition of available food items. This “protein-leverage effect” has previously been reported for omnivorous and herbivorous taxa, including humans (Simpson and Raubenheimer 2005; Sørensen et al. 2008).

MATERIAL AND METHODS

Field and laboratory methods

We collected data in a lowland subtropical semihumid forest in Departamento Santa Cruz, Bolivia. The study area was located in an undisturbed section of the 1 00 000 ha forestry concession “La Chonta” where the average annual precipitation and temperature are 1570 mm and 25 °C, respectively. Within the territory (360–400 ha) of the study community of spider monkeys (*A. chamek*) we established a network of trails, along which daily follows of FAs were conducted as well as monthly phenological surveys and 1 vegetation survey.

Observations of 15 FAs are included in this analysis: 7 adult males and 8 adult females. We did not weigh our FAs as this study was noninvasive. However, male and female spider monkeys are generally of similar body weight (7.5–9 kg) (Smith and Jungers 1997; Karesh et al. 1998). We conducted continuous

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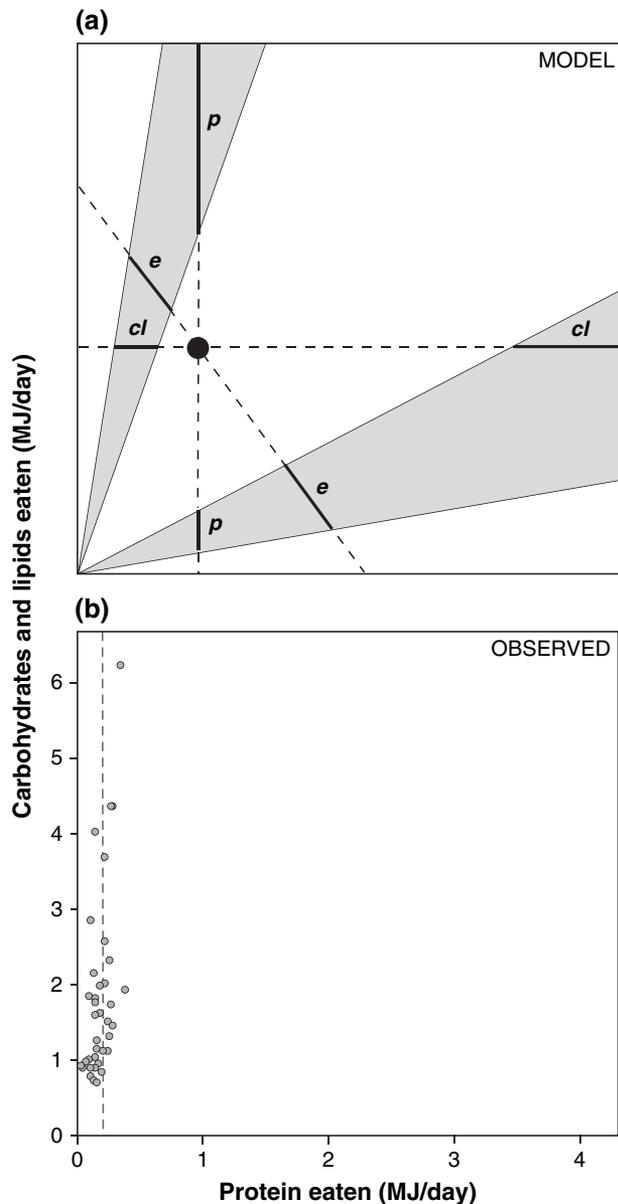


Figure 1

Predicted and observed outcomes of diet selection. (a) A model using the Geometric Framework to represent potential outcomes when animals are fed diets containing different ratios of protein (P) versus carbohydrates (C) and lipids (L) (Simpson et al. 2003). When animals are free to choose foods representing the entire spectrum of P:(C + L) ratios, 2 outcomes are plausible: 1) daily nutritional intakes fall along line *e* due to energy maximization subject to constraints or 2) daily intakes converge around a point in nutritional space (dot) due to target regulation through nutrient balancing. Lines emanating from the origin represent “food rails” that indicate the food’s ratio of P:(C + L). When animals are restricted to diets containing limited amounts of either P or C + L (shading), 3 outcomes are plausible: 1) total energy intake is prioritized (intake points fall along line segments *e*); 2) C + L intake is prioritized (segments *cl*), or 3) protein intake is prioritized (segments *p*). (b) Each point represents the daily endpoint in nutritional space of an FA in this study (38 daily follows). The vertical line, which represents mean protein intake, fits protein prioritization in Figure 1a. Protein intake data indicate available protein and account for any protein bound by tannins (see Methods). There were no significant differences in macronutrient intake between sexes or individuals (see Results).

observations of the same FA from dawn to dusk. We followed each FA for at least 1 whole day each month. In this analysis, we only include days where the FA was successfully followed all day; all feeding events were documented in detail, and relevant analyses existed for every food item consumed ($n = 38$). Females were represented on 26 of the 38 days. The number of days was fairly equal among the 15 different individuals. Both rare and common food items (fruit, leaves, flowers, stems, etc.) were eaten by FAs during the included days. Insects were very rarely observed to be eaten during the study (0.007% of all observed feeding events) and were not part of the 38 included daily diets.

We collected data continuously on the FA’s activities and noted the exact start and end time of each feeding event. Feeding rates (number of items ingested/minute) were documented for all food types, using a stopwatch, when the FA or other individuals were in clear view and were eating continuously without taking a break. For each feeding rate observation, we also noted the type and particular part of the item ingested.

We identified and tagged all plants from which the monkeys were eating and collected food items from these marked feed trees. Whenever possible, we collected items from multiple feed trees of the same species, thereby incorporating between-tree and between-season variation in nutritional content (Chapman et al. 2003; Worman and Chapman 2005). We dried samples in a drying oven (40–50 °C), and later transported samples to The Australian National University. Of the 84 different food items observed to be eaten by FAs during the study, 69 were collected in sufficient amounts to be ground and analyzed (FAs spent <1% of their total feeding time eating items we did not subject to chemical analysis). Many of these food items were divided into their separate components (pulp, husk/wall, and seed) making the final sample size for analysis 106.

We used near infrared reflectance spectroscopy (NIRS) (Foley et al. 1998) to estimate the concentrations of total nitrogen, lipid, starch, neutral detergent fiber (NDF), ash, polyethylene glycol (PEG)-binding capacity, and in vitro digestible N in all food items ($n = 106$). We could not obtain acceptable calibrations to estimate water-soluble carbohydrates (WSC) with NIRS and therefore used directly assayed chemical values for this measure. We used standard procedures for NIRS analysis (Anon 1995) with a FOSS 6500 spectrophotometer (for calibration and cross validation statistics see Supplementary Material Table 1). We selected a representative subset of samples for the calibration data set and analyzed these sub-samples chemically. We carried out all chemical assays in duplicate. We used the Kjeldahl procedure for assaying total N and petroleum spirit extraction in a Soxhlet apparatus for total lipid. We analyzed WSC and starch using the anthrone reaction and a Megazyme Total Starch kit, respectively, as described elsewhere (Lawler et al. 2006). Starch and WSC values are summed and presented as total nonstructural carbohydrates (which in this paper is referred to as “carbohydrates” or “C”). We measured NDF on the residue from lipid (L) analyses using the ANKOM filter bag method (Van Soest et al. 1991; Komarek 1994). To obtain a measurement of tannin concentration that is relevant to the animal, we assayed tannins as the amount of PEG bound per unit dry matter using a method based on that of Silanikove et al. (1996). We estimated the effect of tannins on nitrogen availability using the in vitro digestion procedure described by De Gabriel et al. (2008). We thus estimated available protein as $(\text{total N} \times \text{in vitro N digestibility}) \times 6.25$ (in this paper referred to as “protein” or “P”). We calculated the energetic value of the above nutrient fractions assuming the conventional conversion values of 0.0377 MJ per g lipid, 0.0167 MJ per g crude protein and 0.0167 MJ per g nonstructural carbohydrate (NRC 1989).

Data analysis

When the absolute number of ingested items was not recorded for an event, we multiplied the ingestion time by the feeding rate that most closely matched the corresponding event (see Supplementary Material). We multiplied the nutrient content per food item (g) by the estimated number of items ingested at each event, to obtain a total amount of each nutrient gained from each type of item. We summed all daily events to obtain the daily nutrient intake. We applied the geometric framework as described in Simpson and Raubenheimer (1995) and Raubenheimer and Simpson (1997). The main sources of non-nitrogenous energy are carbohydrates and lipids, with lipids being approximately twice as energy dense as carbohydrates. Lipids comprised only 13.8% of nonprotein energy intake and 12.5% of total energy intake. When plotted separately against protein intake, C and L contributed similarly to the nonprotein energy curve, and for the purpose of this analysis can thus be regarded as interchangeable energy sources (Ruohonen et al. 2007). We therefore combined C and L into one axis (nonprotein energy). For uniformity, protein was plotted in energy units, although mass units could also have been used. Statistical analysis was performed using analysis of variance, principal component regression and linear regression (alpha level = 0.05; for more detail see Supplementary Material).

RESULTS

The food items eaten by *A. chamek* contained 0.9–28% available protein (hereafter referred to as protein, P), 1.8–72% carbohydrates (C) and 0–75% lipids (L) (all % of dry matter). There was no significant difference between sexes in daily nutritional intake either in terms of amount or composition (PC1: $R^2 = 0.004$, $P = 0.857$; PC2: $R^2 = 0.007$, $P = 0.562$; Supplementary Material). Furthermore, there was no significant difference in macronutritional intake between individual FAs (P: $P = 0.843$; C + L: $P = 0.945$). Thus, the more female follow days included in the analysis did not bias the interpretation of nutritional intakes.

Monkeys maintained their daily intake of protein at a mean of 0.19 MJ (± 0.01 SE), whereas nonprotein energy (carbohydrates and lipids) varied between 0.7 and 6.2 MJ (mean 1.82 MJ ± 0.82 SE; Figure 1b). The variation in daily intake of nonprotein energy was significantly related to the availability of ripe fruit in the spider monkey territory (Supplementary Material Table 3). Intake of daily protein (Figure 1b) fits the prediction of the protein-leverage hypothesis (line p in Figure 1a): P was regulated more tightly than either C or L. As the protein concentration in the diet increased, the intake of C and L decreased hyperbolically, following the prediction of the protein-leverage hypothesis (Figure 2).

Spider monkeys did not specifically select either the most energy-rich or the most protein-rich foods that were abundant in the environment (Figure 3). Furthermore, protein intake was not related to the percentage of nonfruit items in the diet (i.e., leaves and flowers: $R^2 = 0.004$, $P = 0.719$), nor to temporal fluctuations in the abundance of different food items (Supplementary Material Table 3).

Daily nutrient intake was generally not influenced by the concentration of tannins in the food. Both leaves and fruit consumed by spider monkeys contained tannins (leaves, mean 12% ± 0.55 SE; fruit, mean 10% ± 1.33 SE; tannin concentration measured as % bound polyethylene glycol). Nevertheless, there was no relationship between dietary tannin and intake of protein ($R^2 = 0.014$, $P = 0.473$) or nonprotein energy sources ($R^2 = 0.000$, $P = 0.994$). However, the data suggest that C + L intake was reduced when tannin levels were higher than 16% by dry mass and protein levels in the diet were low (Figure 4).

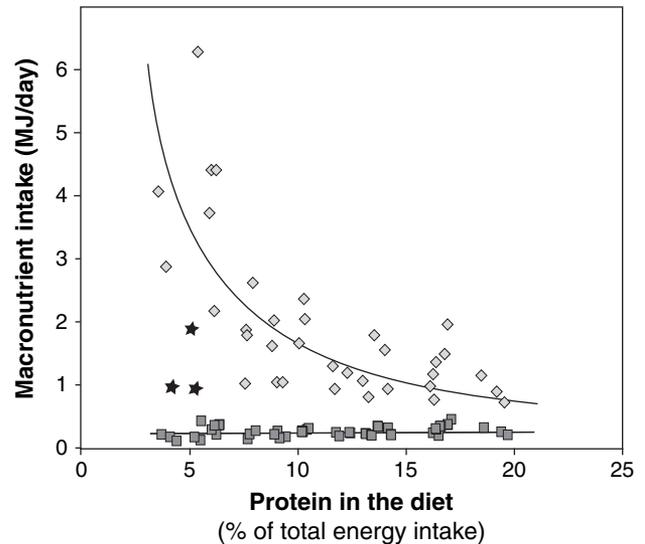


Figure 2

Macronutrient intake across varying diets. Relationship between observed intake of protein (squares) and nonprotein energy (C + L, diamonds) across a range of diets with varying proportions of protein, in relation to the expected C + L intake (hyperbolic trend line) assuming complete protein leverage (i.e., intake of protein is maintained constant when diet composition varies). We used data from 38 focal days to calculate the expected C + L intake according to the equation $C + L = (P_t/p) - P_t$; where P_t is the target intake of protein and p is the proportion of protein of total energy intake (Simpson et al. 2003). We assumed that the observed mean protein intake approximated the physiological target intake P_t . Absolute protein intake did not vary across diets ($R^2 = 0.034$, $p = 0.287$, horizontal trend line). If monkeys had instead prioritized daily energy intake, then the sum of P + C + L versus %P would produce a horizontal line. Stars indicate days when dietary tannin was exceptionally high and likely caused C + L intake to be lower than expected (see Figure 4 and Supplementary Material). C + L intake varied significantly across diets including or excluding days with high dietary tannins ($n = 38$, $R^2 = 0.313$, $P < 0.001$; $n = 35$, $R^2 = 0.452$, $P < 0.001$).

Constraining factors such as gut limitations and feeding time could not explain our results. FAs were observed to consume up to 2.4 kg fresh matter per day but averaged 1.0 kg/day (SE 0.08 kg). There was no relationship between total food and fiber intake ($R^2 = 0.007$, $P = 0.620$). Daily feeding time varied between 52 and 400 min (mean 173 ± 12.5 SE), and there was no relationship between daily feeding time and protein intake ($R^2 = 0.039$, $P = 0.209$) or C + L intake ($R^2 = 0.047$, $P = 0.167$).

DISCUSSION

Our results fit the prediction of the protein-leverage hypothesis: Spider monkeys regulated their daily intake of available protein much more tightly than they did either carbohydrates or lipids, and this regulation disproportionately influenced total energy intake. The protein-leverage hypothesis predicts that in the most extreme case, nonprotein energy intake (i.e., intake of nonstructural carbohydrates plus lipids; C + L) will decrease hyperbolically with an increasing proportion of protein in the diet, whereas protein gain will remain constant (Simpson and Raubenheimer 2005). The observed selection of nutrients by spider monkeys closely resembled the pattern expected by this model (Figure 2).

Frugivorous atelines are frequently observed to preferentially select and consume large volumes of fruit that are rich in lipids and soluble carbohydrates (Castellanos 1995; Dew

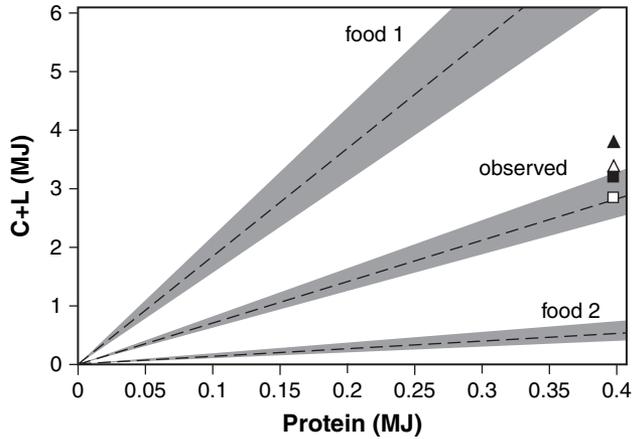


Figure 3

Observed intake versus macronutrient balance of food groups. Comparison of the mean observed intake trajectory across all observation days (“observed”) with food rails representing the mean of the lowest (“food 1”) and the mean of the highest P:(C + L) foods (“food 2”) that were sampled by the monkeys on each day and were abundant in the home range (shaded areas = 95% confident intervals). The 3 means were significantly different from each other ($P < 0.001$). The low P:(C + L) food group had significantly higher total energy content ($P < 0.001$) and lower protein content ($P < 0.001$) than the high P:(C + L) food group. Also indicated are the positions of 4 species of ripe fruit that appear to have balanced macronutritional compositions in relation to the animals’ average intake trajectory (▲: *Ficus boliviana*, △: *Socratea exorrhiza*, ■: *Jacaratia spinosa*, and □: *Celtis iguanea*). The observed intake was not a product of individuals consuming large quantities of *F. boliviana*, their staple food (Felton et al. 2008). The intake trajectory of days when figs were absent from their diet was not significantly different from the overall mean ($n = 12$, $P = 0.155$). This supports the conclusion that *F. boliviana* and other foods indicated in the figure are indeed nutritionally balanced foods for these primates.

2005; Di Fiore et al. 2008). Due to this apparent preference for energy-dense foodstuffs, coupled with the highly energetic lifestyles of these frugivores, energy has been suggested to be the primary driver behind their food choice (Rosenberger and Strier 1989; Strier 1992; Di Fiore and Rodman 2001). If

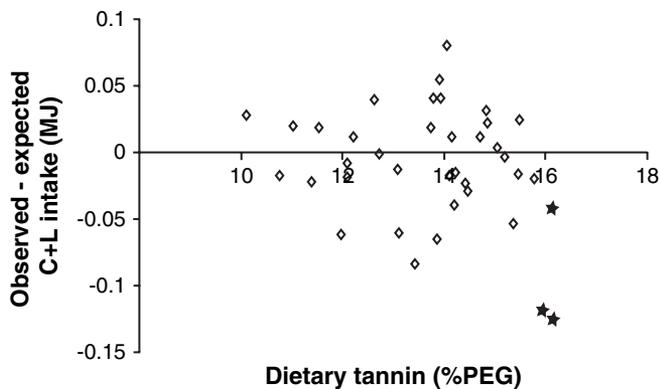


Figure 4

The perpendicular distance between the observed and expected C + L intakes for each day (which are both depicted in Figure 2) in relation to tannin content in the corresponding diet. Tannin concentration was measured as % bound PEG. All 3 data points that fall above 16% PEG (denoted as stars in this figure and Figure 2) deviate significantly more from the expected C + L intake than the remaining points ($P < 0.001$) and represent days when protein constituted $\leq 5\%$ of total energy intake.

this were true for the monkeys in our study, then observed data points would fall along line *e* instead of line *b* in Figure 1. Furthermore, if energy was prioritized, the daily intake trajectories would track the most energy-rich foods that were abundant in the environment. This was not the case, as substantial portions of more protein-rich foods were included in the diet (Figure 3). Neither, do the data suggest the goal of protein maximization, as would be indicated if the intake trajectories tracked the most protein-rich foods available. Rather, the monkeys selected foods spanning a range of protein:nonprotein energy ratios and regulated their intake so as to achieve a constant daily protein gain. As a consequence, the variation in the protein:nonprotein energy balance of the selected foods translated into variable nonprotein and total energy gain. Furthermore, protein intake was not related to the percentage of nonfruit items in the diet (i.e., leaves, seeds, flowers, etc.), nor to temporal fluctuations in the abundance of different food items throughout the field study that spanned distinct periods of abundance and scarcity of different food resources (Felton et al. 2008). This indicates that individuals reached their protein target regardless of the food choices available. To maintain a stable intake of protein, spider monkeys consumed large amounts of C and L when protein content in the food was low—for example, when their diet consisted entirely of ripe fruit, and consumed much less C and L when feeding on items rich in protein.

PSMs may have played a role in determining which foods spider monkeys chose to eat and which to reject. However, on the scale of nutrient intake gained from selected foods, our results demonstrate that PSMs did not dominate the patterns observed. Tannins, which occur in both leaves and fruit consumed by spider monkeys, greatly reduce the availability of protein (Robbins et al. 1987). Our data suggest that although there was no relationship between dietary tannin and intake of protein or nonprotein energy sources, C + L intake was reduced when tannin levels were higher than a certain threshold and protein levels in the diet were low (Figure 4). This finding is in keeping with other research showing that tannins have a disproportionate effect on food intake when protein levels in food are low (Simpson and Raubenheimer 2001; Villalba and Provenza 2005).

When explaining feeding patterns of animals, some branches of nutritional ecology (e.g., optimal foraging theory, Schoener 1971) emphasize constraining factors such as gut limitations and time spent feeding (Mattson 1980). These constraints cannot explain our results. The average daily fresh matter intake by FAs was much lower than the maximum observed, which indicates that volumetric constraints could not explain the data. Nor was there a relationship between total food and fiber intake, or between daily feeding time and nutrient intake. Finally, it is highly unlikely that an upper limit to protein intake limited total energy intake. This is because toxic effects of overconsumption are noticeable only when animals ingest amino acids at much higher amounts than those encountered in our study (Harper et al. 1970; De Gabriel et al. 2002).

CONCLUSIONS

Data reported here show, for the first time, that protein-dominated macronutrient balancing best describes the nutritional strategy of a nonhuman primate. Although the daily protein gain of the wild spider monkeys was comparatively low, the amount of protein ingested daily was maintained remarkably stable despite significant variation in food composition. Such findings can contribute to the management of wild populations, by characterizing conservation problems in terms of nutrient landscapes. For instance, figs of the commercial

timber species *Ficus boliviana* were a major food source for our study animals (Felton et al. 2008), and also appear to be a nutritionally balanced food for spider monkeys (Figure 3). Nutritionally balanced food sources that are used extensively by a wild population may need special attention in conservation planning, for example, as the basis for regulating the logging of certain tree species, or selecting target tree species for establishment in vegetation restoration. A more complex picture also emerges from our analysis, in which the monkeys are able to regulate their nutritional state through selecting complementary combinations of other foods. A challenge therefore is to conserve a diversity of foods that provide foragers with access to the optimal region within nutrient space (Figure 1a).

Our findings are potentially of interest in understanding the evolutionary and ecological origins of human susceptibility to obesity. Human obesity and associated disease is commonly attributed to a mismatch between modern diets rich in carbohydrates and lipids, and phenotypes that evolved in Paleolithic nutritional ecologies where diets were relatively high in protein and fiber (Eaton et al. 1999; Cordain et al. 2000). It has been proposed that due to the scarcity of physiologically available, simple carbohydrates and fats in the ancestral diet, humans have evolved strong taste preferences for these macronutrients (Galef 1996; Simpson and Raubenheimer 2005). According to the “protein-leverage hypothesis,” in order to maintain relative constancy of absolute protein intake, humans are predisposed toward diluting the protein in the modern diet through increased consumption of highly palatable foods rich in fats and carbohydrates, which leads to overconsumption of energy (Simpson and Raubenheimer 2005). The regulatory dominance of protein over nonprotein energy intake therefore appears to play a central role in human obesity (Simpson and Raubenheimer 2005). Our demonstration that protein similarly dominates over total daily energy intake in a frugivorous platyrrhine primate is intriguing. Why protein should be regulated is clear: It provides the primary source of dietary nitrogen for growth. However, its dominance over daily energy intake in a population living in its natural habitat suggests that the costs of eating either excesses or deficits of carbohydrates and fats on a given day to ensure ingesting the target amount of protein are small in relation to the costs of failing to meet the protein intake target (Cheng et al. 2008). A likely contributor to this cost asymmetry is the fact that excess energy can readily be stored and drawn on in days of negative energy balance, whereas no such buffer exists for protein.

The similarity in the regulatory pattern of protein intake between humans and spider monkeys suggests that the genetic origins of an important contributor to human susceptibility to obesity could be far older than the Paleolithic. This is consistent with the hypothesis that human micronutrient requirements have a pre-Paleolithic origin (Milton 2003b). Anatomical, physiological, and paleontological evidence support the general consensus that the ancestral form giving rise to the human lineage (*Homo*) was markedly herbivorous, similar to modern apes and monkeys (Milton 2000, 2003b). Furthermore, the extensive homology of the “obesity gene product” among vertebrates also suggests that susceptibility to obesity may have ancient origins (Zhang et al. 1995). We hypothesize that although the gut morphology of *Homo* has evolved to digest higher quality food compared with apes and monkeys (Milton 2003a), the mechanisms for macronutrient regulation have remained relatively unchanged. At present, we are unable to distinguish between this explanation and the possibility that protein-dominated macronutrient balancing evolved independently in *Homo* and *Ateles*. We recommend that further comparative studies into nutritional strategies of wild nonhuman primates, representing a wide range of diets

and phylogenetic relatedness to humans, are conducted to elucidate this issue.

The findings of this study may also be of value for the management of captive primate populations, in which obesity and associated health problems are common (Terranova and Coffman 1997; Videan et al. 2007). Primate species, such as *A. chamek*, that have evolved in environments with significant annual or supra-annual fluctuations in food availability deal with excess energy consumption primarily by storing fat (Leighton 1993; Knott 1998; Di Fiore and Rodman 2001; Schwitzer and Kaumanns 2001). Our results suggest that an adjustment of the macronutritional balance of diets as a means to manage human obesity might similarly be an option for mitigating obesity in captive primates.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.beheco.oxfordjournals.org/>

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