Potential Applications of Urinary C-Peptide of Insulin for Comparative Energetics Research

Diana S. Sherry* and Peter T. Ellison

Department of Anthropology, Harvard University, Cambridge, MA 02138

ABSTRACT

The study of comparative energetics offers a valuable way to identify broad ecological principles and assess the functional significance of energetic adaptations during the course of evolution. Yet, the quantification of energetic status for nonhuman primates under natural conditions remains one of the most challenging aspects of comparative energetics research. Here, we report on the development of a noninvasive field method for measuring energetic status in great apes, humans, and possibly other nonhuman primates. Specifically, we have explored measurement of a urinary metabolite of insulin (C-peptide) as a physiological marker of energetic condition in chimpanzees and orangutans. We performed three validation studies and successfully measured C-peptide in urine samples from captive chimpanzees, wild chimpanzees, and wild orangutans. Urinary C-peptide measures gave indications of being a reliable signal of energetic status in both species. For chimpanzees and orangutans in the wild, baseline urinary C-peptide levels were higher during periods of fruit abundance than periods of low fruit availability. Urinary C-peptide levels were also higher for well-fed captive chimpanzees compared with wild chimpanzees. Although sample size was small, top-ranking male chimpanzees showed higher C-peptide levels in the wild than low-ranking males only during the period of fruit abundance. These preliminary results indicate that further development of the urinary C-peptide method could expand opportunities to quantify energetic condition for great apes in the wild and generate new data for comparative research. We highlight specific applications for studying great ape reproduction as well as the nutritional ecology of human foragers. Am J Phys Anthropol 000:000–000, 2007. © 2007 Wiley-Liss, Inc.

The ability of organisms to sequester and utilize energy from the environment constitutes one of the most fundamental parameters in evolution. Energy available for growth, maintenance, and reproduction influences mortality and fertility patterns as well as life history attributes (Williams, 1966; Western, 1979; Charnov and Berri- gan, 1993). The study of comparative energetics in great apes and humans offers a useful way to evaluate energetic principles shaping human evolution (Leonard and Robertson, 1997; Aiello and Key, 2002). Yet, the ability to test relationships between ecological variables and energetic adaptations rests on effective means to measure energetic outcomes. One of the major obstacles hindering comparative energetics research has been the challenge of acquiring empirical data sets under natural conditions. In general, energetic status has been extremely difficult to measure for nonhuman primates in the wild.

The most common field method used to quantify energetic condition involves the use of tranquilizers to collect body weight data (Mori, 1979; Bercovich, 1987; Goldizen et al., 1988; Altman et al., 1993; Richard et al., 2000; Kurita et al., 2002). Other approaches have included visual assessment of body mass (Berman and Schwartz, 1988) and estimates of energy intake and expenditure (Knott, 1998; Koenig and Borries, 2001). At some of the major chimpanzee field sites, researchers have gathered body weight data noninvasively by enticing animals up a rope with a scale attached to retrieve pieces of banana or sugarcane (Goodall, 1986; Uehara and Nishida, 1987; Boesch and Boesch-Achermann, 2000; Pusey et al., 2005).

In this article, we report on the development of a novel field method for quantifying energetic condition in great apes. We then highlight promising applications of this technique for comparative research. Specifically, we have explored measurement of a urinary metabolite of insulin (C-peptide) in chimpanzees and orangutans as a physiological indicator of energetic status. Insulin is the main metabolic hormone associated with the fed state in humans. Although some aspects of reliability remain to be resolved, our preliminary results suggest that urinary C-peptide can provide a potential biomarker of energy balance—the difference between caloric intake and expenditure—in great ape species. Continued verification of the urinary C-peptide method could establish a useful, noninvasive tool and standardized variable for comparative energetics research.

BACKGROUND

Steady improvements in the collection and analysis of urinary metabolites over the past two decades have brought a major technological advance to primate field studies, especially in the area of reproductive endocrinology (Hodges et al., 1979; Lasley et al., 1980; Andelman et al., 1985; Munro et al., 1991; Shideler et al., 1995; Knott, 1997; Whitten et al., 1998; Muller and Lipson, 2003; Emery Thompson, 2005). Nonetheless, a reliable

*Correspondence to: Diana S. Sherry, Department of Anthropology, 11 Divinity Ave, Harvard University, Cambridge, MA 02138, USA. E-mail: dsherry@fas.harvard.edu

Received 18 November 2005; accepted 16 November 2006

DOI 10.1002/ajpa.20562
Published online in Wiley InterScience (www.interscience.wiley.com).
urinary indicator of energetic status has yet to be determined. Urinary cortisol has been measured successfully in wild primates (van Schaik et al., 1991; Robbins and Czekala, 1997; Muller and Wrangham, 2004) but remains problematic as an energetic signal because of potentially confounding psychosocial factors (Sapolsky, 1992; Pollard, 1995; Abbott et al., 2003). Ketone bodies, the by-products of fat catabolism, have been detected successfully in urine samples from wild orangutans (Knott, 1998) but not from wild chimpanzees (Wrangham, pers. comm.).

In humans, insulin functions as a major metabolic hormone. Insulin regulates glucose homeostasis, facilitates glucose uptake by tissues, and promotes the storage of surplus energy as glycogen and fat (Foster and McGarry, 1992). Baseline (fasting) insulin levels tend to track changes in energy balance as the body shifts between states of energy storage and utilization (Andersson et al., 1991; Koivisto et al., 1992; Doucet et al., 2000). Although body weight tends to correlate with sustained shifts in energy balance over the long term, shifts in energy storage versus utilization often occur before, and sometimes in the absence of, any appreciable change in body weight or fat level (Doucet et al., 2000; Jasienska and Ellison, 1998, 2004). Insulin offers a potentially more sensitive indicator of energy dynamics than body weight measures because insulin itself regulates the synthesis or breakdown of energy reserves.

Compared to humans, relatively little is known about insulin dynamics in great apes and other nonhuman primates. In captivity, chimpanzees can develop pathologies similar to humans such as obesity-related adult-onset diabetes and impaired glucose tolerance (Rosenblum and Drash, 1981; Stein et al., 1995). Extensive laboratory studies of rhesus macaques, however, provide a well-documented model of insulin dynamics in a nonhuman primate and point to a broad similarity between humans and an Old World monkey (Kemnitz et al., 1988; Wolden-Hanson et al., 1993; Kemnitz et al., 1994). We suggest that similar dynamics probably operate in great ape species as well, given their close phylogenetic relationship to humans. In addition, a field study of wild baboons showed that insulin measures indicated a biologically significant difference in energetic status for males that did not show up as a statistically significant difference in body weight (Kemnitz et al., 2002).

'C-peptide' of insulin refers to the connecting chain of amino acids cleaved during pancreatic conversion from proinsulin to insulin and released into the bloodstream as a by-product. A number of attributes make C-peptide an ideal metabolite for measuring insulin. The conversion process results in an equimolar amount of insulin and C-peptide such that C-peptide gives an indirect indication of insulin production (Aro, 1985). Unlike insulin, C-peptide remains biologically inert in the bloodstream and a consistent fraction of production (about 5%) is excreted intact in the urine (Kitabchi et al., 1977; Kruzsynska et al., 1987). Urinary clearance of C-peptide also parallels the rate of production, allowing an integrated measure of change in baseline insulin levels from urine. In short, urinary C-peptide offers a noninvasive index of baseline insulin production.

C-peptide has been used widely in clinical studies, with laboratory protocols for measurement in blood and urine well established for humans (Bonser and Garcia-Webb, 1984; Gjessing et al., 1989; Stein et al., 1994; Miura et al., 1996; Sameshima et al., 1999). Nonetheless, urinary C-peptide measurement has been only recently applied to an anthropological field study on humans (Valegga and Ellison, 2001; Ellison and Valegga, 2003). Laboratory studies of rhesus macaques have shown that urinary C-peptide also provides a reliable index of insulin production in an Old World primate (Wolden-Hanson et al., 1993; Kemnitz et al., 1994). In rhesus macaques, the amino acid sequence for C-peptide differs from humans by only one amino acid (Naithani et al., 1984), facilitating use of immunoassay protocols developed for humans. Although few amino acid sequences have been determined for other nonhuman primates, the structure of proinsulin appears highly conserved in mammalian evolution (Snell and Smyth, 1975). Presumably, C-peptide assays developed for humans could be used to measure C-peptide in great apes as well, given that great apes are even more closely related to humans than are rhesus macaques.

RESEARCH AIMS

We conducted three preliminary studies to assess whether urinary C-peptide could provide a physiological marker of energetic status in great apes. First, we set out to verify whether measurement of C-peptide in urine would be reliable in chimpanzees. Urinary C-peptide had not been validated previously in chimpanzees and, without further assessment, we could not rule out possible species differences in the degree of protein degradation or fragmentation of the C-peptide molecule during urinary clearance. To address this issue, we performed a basic validation study using matched blood and urine samples from captive chimpanzees.

Second, we examined whether urinary C-peptide would convey an accurate signal of energetic condition for chimpanzees in the wild. If baseline levels reflected energetic status, we expected to find the following two relationships: 1) urinary C-peptide levels would be higher on average in well-fed captive chimpanzees than in wild chimpanzees, and 2) urinary C-peptide levels in wild chimpanzees would be higher on average during periods of fruit abundance than during periods of low fruit availability. We also explored the relationship between urinary C-peptide level and dominance rank for male chimpanzees in the wild.

For our third study, we examined urinary C-peptide as a potential biomarker of energetic status for orangutans in the wild. We set out to determine: 1) the feasibility of measuring C-peptide from urine samples preserved on filter paper and 2) whether urinary C-peptide showed evidence of being an energetic signal consistent with other, independent field assessments of energetic status in the wild.

METHODS

Captive chimpanzee study

We used 29 matched serum and urine samples from 14 males and 15 females collected from captive chimpanzees at the Yerkes Regional Primate Center. Males ranged from 15- to 28-years-old with body weights from 55 to 92 kg. Females ranged from 12- to 39-years-old with body weights from 49 to 73 kg. Females showed a significant relationship to humans. In addition, a field study of wild baboons showed that insulin measures indicated a biologically significant difference in energetic status for males that did not show up as a statistically significant difference in body weight (Kemnitz et al., 2002).

'C-peptide' of insulin refers to the connecting chain of amino acids cleaved during pancreatic conversion from proinsulin to insulin and released into the bloodstream as a by-product. A number of attributes make C-peptide an ideal metabolite for measuring insulin. The conversion process results in an equimolar amount of insulin and C-peptide such that C-peptide gives an indirect indication of insulin production (Aro, 1985). Unlike insulin, C-peptide remains biologically inert in the bloodstream and a consistent fraction of production (about 5%) is excreted intact in the urine (Kitabchi et al., 1977; Kruzsynska et al., 1987). Urinary clearance of C-peptide also parallels the rate of production, allowing an integrated measure of change in baseline insulin levels from urine. In short, urinary C-peptide offers a noninvasive index of baseline insulin production.

C-peptide has been used widely in clinical studies, with laboratory protocols for measurement in blood and urine well established for humans (Bonser and Garcia-Webb, 1984; Gjessing et al., 1989; Stein et al., 1994; Miura et al., 1996; Sameshima et al., 1999). Nonetheless, urinary C-peptide measurement has been only recently applied to an anthropological field study on humans (Valegga and Ellison, 2001; Ellison and Valegga, 2003). Laboratory studies of rhesus macaques have shown that urinary C-peptide also provides a reliable index of insulin production in an Old World primate (Wolden-Hanson et al., 1993; Kemnitz et al., 1994). In rhesus macaques, the amino acid sequence for C-peptide differs from humans by only one amino acid (Naithani et al., 1984), facilitating use of immunoassay protocols developed for humans. Although few amino acid sequences have been determined for other nonhuman primates, the structure of proinsulin appears highly conserved in mammalian evolution (Snell and Smyth, 1975). Presumably, C-peptide assays developed for humans could be used to measure C-peptide in great apes as well, given that great apes are even more closely related to humans than are rhesus macaques.

RESEARCH AIMS

We conducted three preliminary studies to assess whether urinary C-peptide could provide a physiological marker of energetic status in great apes. First, we set out to verify whether measurement of C-peptide in urine would be reliable in chimpanzees. Urinary C-peptide had not been validated previously in chimpanzees and, without further assessment, we could not rule out possible species differences in the degree of protein degradation or fragmentation of the C-peptide molecule during urinary clearance. To address this issue, we performed a basic validation study using matched blood and urine samples from captive chimpanzees.

Second, we examined whether urinary C-peptide would convey an accurate signal of energetic condition for chimpanzees in the wild. If baseline levels reflected energetic status, we expected to find the following two relationships: 1) urinary C-peptide levels would be higher on average in well-fed captive chimpanzees than in wild chimpanzees, and 2) urinary C-peptide levels in wild chimpanzees would be higher on average during periods of fruit abundance than during periods of low fruit availability. We also explored the relationship between urinary C-peptide level and dominance rank for male chimpanzees in the wild.

For our third study, we examined urinary C-peptide as a potential biomarker of energetic status for orangutans in the wild. We set out to determine: 1) the feasibility of measuring C-peptide from urine samples preserved on filter paper and 2) whether urinary C-peptide showed evidence of being an energetic signal consistent with other, independent field assessments of energetic status in the wild.

METHODS

Captive chimpanzee study

We used 29 matched serum and urine samples from 14 males and 15 females collected from captive chimpanzees at the Yerkes Regional Primate Center. Males ranged from 15- to 28-years-old with body weights from 55 to 92 kg. Females ranged from 12- to 39-years-old with body weights from 49 to 73 kg. Ideally, a validation study based on paired comparisons between hormonal measures in serum and urine would involve intravenously monitored serum levels over a 24-h period and collection of the total volume of urine excreted. Since the 24-h protocol is highly invasive and
was not a feasible option in this case, we made use of matched serum and urine samples collected at Yerkes during the routine, annual physical exam following an overnight fast. Blood samples were drawn intravenously and urine samples were removed from the bladder by catheter. We expected these samples to show a more moderate correlation than the 24-h collection procedure because instantaneous serum samples tend to reflect immediate, short-term circulating levels whereas urine samples tend to integrate hormonal levels over the time between urinations (Cook and Beastall, 1987).

We measured C-peptide with a commercially available radioimmunoassay kit from Diagnostic Systems Laboratories (Webster, TX) designed to measure C-peptide of insulin in human serum, plasma, and urine (DSL-7000). Each kit cost $120 plus shipping and measured 40 samples in duplicate. The reference norms for human adult males and females provided by the assay manufacturer ranged from 1.1 to 3.2 ng/ml for 12-h fasting serum samples and below 140 µg/day for urine samples (multiplied by the total volume of urine excreted over a 24-h period and divided by 1,000 to convert from ng to µg/day).

During an earlier phase of development, we had acquired urine samples from captive chimpanzees at New Iberia Primate Research Center and ran a series of dilution experiments to test the initial feasibility of measuring C-peptide in urine. We had determined that unless the urine sample was extremely concentrated (showed relatively high creatinine values; see below), the dilution factor called for by the assay manufacturer for humans (1:10) was not necessary for chimpanzee samples to register properly on the standard dose-response curve. All measures of urinary C-peptide were expressed per unit creatinine present in the sample. The level of creatinine, a protein product of muscle maintenance activity excreted at a constant rate, standardizes urine samples for differences in water content and urinary clearance period (Narayanan and Appleton, 1980). Creatinine was measured based on colorimetric determination by the Jaffe reaction (Taussky, 1954). All assays were performed at the Reproductive Ecology Laboratory at Harvard University.

**Wild chimpanzee study**

As part of ongoing field research at Kibale National Park, Uganda, urine samples have been collected systematically from the Kanyawara chimpanzee community since 1997. Our study of C-peptide in wild chimpanzees focused on the year 1998 to make use of urine samples available for exploratory analyses from prior dissertation research (Muller, 2002) in conjunction with an established data set on feeding conditions from the same year (Sherry, 2002).

Protocols for field collection, storage, and transportation of urine samples have been described elsewhere (Knott, 1997; Muller and Wrangham, 2004). In the wild, chimpanzees typically urinate from a tree upon waking in the morning. Sample collection involved catching the urine with a clean plastic “net” attached to a pole or on clean plastic sheets placed on the ground. Urine was then transferred to collection tubes with a disposable pipette, sealed, and labeled. Collection tubes were frozen upon arrival at the field station laboratory 1–10 h later, until delivered on dry ice to Harvard University.

Sample selection for urinary C-peptide analyses included the following three criteria: 1) collection time in the field had to occur prior to 0730 h to ensure waking or baseline values, 2) sufficient sample volume had to remain from previous analyses to allow measurement of C-peptide in duplicate as well as creatinine measurement, and 3) urine samples had to come from either the high or low fruiting period for adults of both sexes.

Previous analyses of chimpanzee feeding behavior had identified periods of high and low fruit availability for 1998 (Sherry, 2002). At Kanyawara, data collection on feeding behavior involves scan sampling at 15-min intervals during daily follows of chimpanzee groups. On the basis of changes in the relative proportion of fruit in the diet, feeding observations allow an indirect way to track fruit availability over time. As preferred fruits become available in the environment, they are typically eaten in greater quantities. A monthly feeding index was calculated for each of the seven main fruits in the chimpanzee diet, three of which are succulents and four of which are figs (Wrangham et al., 1996). Following Leighton (1993), each monthly feeding index was computed as the ratio between the number of feeding observations on the particular (ripe) fruit and the total number of feeding observations for the month. The highest fruiting period for 1998 was in April and May, with 63.2% of the diet comprised of ripe fruit (mostly figs). The lowest fruiting period took place in October and November, with 24.3% of the diet comprised of ripe fruit. A total of 43 urine samples, 25 from 6 adult males and 18 from 6 cycling females met the selection criteria above. Urinary C-peptide and creatinine measures were based on the same assay protocols used in the captive chimpanzee study.

**Wild orangutan study**

Samples for urinary C-peptide measurement in wild orangutans came from a field study conducted at Gunung Palung National Park, Indonesia, in the 1990s (Knott, 1999). Sample collection generally followed the same procedures described for chimpanzees in the wild, except that orangutan urine samples were preserved on filter paper rather than frozen. In most cases, sample collection involved placing clean plastic sheets on the ground below the sleeping tree to catch the first void of urine in the morning. Many samples thus represent fasting or baseline values. Urine was then transferred to collection tubes with a disposable pipette and labeled. At the field station, several filter-paper replicates were prepared for each specimen by placing 200 µl of urine onto a 2.5 cm square of filter paper. Samples were then dried thoroughly in a sealed container with silica gel and stored individually in slide protector sheets until frozen upon arrival at Harvard University.

To determine whether the filter paper matrix itself would interfere with measurement of urinary C-peptide, we performed a separate validation study. We used frozen urine specimens from humans and prepared several matched filter-paper samples for each specimen. Replicates were prepared by placing 200 µl of urine onto a 2.5 cm square of filter paper and then allowed to dry in the laboratory overnight. We reconstituted the filter-paper samples by using a hand-held hole punch to remove 2–3 circles from each filter-paper square directly into a test tube to achieve the necessary sample volume per specimen. The punched circles were then eluted overnight in zero standard solution and assayed along with the matched frozen urine samples according to...
the manufacturer’s instructions (DSL-7000). Urinary C-peptide values (ng/ml) were highly correlated between the frozen and matched filter-paper samples ($r = 0.94$; $y = 0.933x + 0.135$, $P = 0.0001$; $n = 12$). In addition, a paired t-test showed no significant difference in mean C-peptide levels between the matched frozen and filter-paper samples (35.4 vs. 32.4 ng/ml, respectively, $P = 0.301$; df = 11). Measurements of urinary creatinine (mg/ml) were also highly correlated ($r = 0.97$; $y = 0.903x + 0.007$, $P = 0.0001$; $n = 12$).

Since it was possible to measure C-peptide reliably in urine samples preserved on filter paper, we proceeded with the C-peptide study in wild orangutans. Our next concern was to determine the appropriate dilution factor in order to select C-peptide to register well on the standard dose-response curve. We limited the number of urine samples to six different specimens in order to examine possible dilution factors. To evaluate C-peptide as an energetic signal as well, we created three paired contrasts between the mast fruiting episode that took place from October 1994 to February 1995 and a subsequent period of low fruit availability. The samples chosen from the mast coincided with the onset of the mast, the peak of the mast, and the final stage of the mast from three different individuals. We then selected samples, two of which contained ketone bodies, from a low fruiting period for the same three individuals. The orangutan urine samples were prepared by removing 2–3 circles from each filter-paper square with a hole punch directly into a test tube to attain sufficient sample volume. Samples were then reconstituted overnight in zero standard solution, diluted at three different concentrations, and measured for urinary C-peptide and creatinine level with the same assay protocols described for the chimpanzee studies.

Data analysis

Data analysis consisted of basic parametric techniques. We performed a linear regression analysis to evaluate the relationship between C-peptide levels in the matched serum and urine samples. For statistical comparisons of mean urinary C-peptide level between two categorical variables, we used two-tailed student’s t-tests. Specific analyses are described further in the Results section. Results with a P-value below 0.05 were considered statistically significant.

RESULTS

Captive chimpanzee study

Although published reference norms for C-peptide in captive chimpanzees have not been established previously, fasting serum levels of insulin have been shown to be roughly equivalent to humans (Steinetz et al., 1996). We found that fasting serum levels of C-peptide were similar to human values as well, ranging from 1.4 to 1.4 ng/ml and no significant sex difference ($n = 29$). Urinary C-peptide levels ranged from 1.7 to 27.8 ng/mg Cr/ml with a mean and standard deviation of 10.6 ± 6.6 ng/mg Cr/ml ($n = 29$). There was no significant correlation between C-peptide and body weight, presumably because all animals were well fed regardless of size. C-peptide showed a slight tendency to decline with age for both sexes—a pattern observed in humans for insulin levels.

C-peptide was also measured successfully in urine samples from wild chimpanzees and gave indications of being a reliable energetic signal. Baseline levels for urinary C-peptide ranged from 0.62 to 13.0 ng/mg Cr/ml with a mean and standard deviation of 4.7 ± 2.9 ng/mg Cr/ml ($n = 43$). As presented in Figure 2, wild chimpanzees showed a significantly lower mean urinary C-peptide level than their captive counterparts (two-tailed t-test, $t = -5.19$, $P < 0.0001$, df = 70).

Figure 1 shows the comparison of mean urinary C-peptide level during the high and low fruiting period. Data analysis included values from males only because sample size was too small to control for variation in female energetic status related to the duration of lactation postpartum. Of the 25 urine samples available from males, 14 came from 5 males during the high fruiting period and 11 came from 6 males during the low fruiting period. Since urine samples representing both fruiting periods did not correspond to the same males in every case, we performed an unpaired t-test (two-tailed) rather than a paired comparison. Mean urinary C-peptide level was significantly higher for males during the high fruiting period than the low fruiting period (4.3 ng/mg Cr/ml vs. 2.2 ng/mg Cr/ml, respectively; $t = -2.11$, $P < 0.05$, df = 23). The greatest range of intraindividual variation occurred in the alpha male and came to 7.24 ng/mg Cr/ml (2.38–9.62) during the high fruiting period. The smallest range of intraindividual variation occurred in the alpha female and came to 4.29 ng/mg Cr/ml (1.26–9.31) during the low fruiting period. Data analysis included values from females only because sample size was too small to control for variation in male energetic status related to the duration of lactation postpartum. Of the 10 urine samples available from females, 7 came from 4 females during the high fruiting period and 3 came from 2 females during the low fruiting period. Since urine samples representing both fruiting periods did not correspond to the same females in every case, we performed an unpaired t-test (two-tailed) rather than a paired comparison. Mean urinary C-peptide level was significantly higher for females during the high fruiting period than the low fruiting period (4.9 ng/mg Cr/ml vs. 2.6 ng/mg Cr/ml, respectively; $t = -2.21$, $P < 0.05$, df = 18). The greatest range of intraindividual variation occurred in the alpha female and came to 7.58 ng/mg Cr/ml (2.38–9.62) during the high fruiting period. The smallest range of intraindividual variation occurred in the alpha male and came to 4.13 ng/mg Cr/ml (1.26–9.31) during the low fruiting period.
variation occurred in a low-ranking male of similar age and came to 2.18 ng/mg Cr/ml (2.58–4.76), also during the high fruiting period.

To explore the relationship between male dominance rank and urinary C-peptide levels further, we compared C-peptide levels for the two highest-ranking males versus the two lowest-ranking males during both the high and low fruiting periods. We found a significant difference in mean urinary C-peptide level between the highest and lowest-ranking males only during the high fruiting period. As shown in Figure 4, mean urinary C-peptide level came to 6.6 ng/mg Cr/ml ($n = 5$ samples) for the highest-ranking males and 3.4 ng/mg Cr/ml ($n = 6$ samples) for the lowest-ranking males ($t = 2.44$, $P < 0.05$, df = 9). In contrast, we found no significant difference in mean urinary C-peptide level between the same highest and lowest-ranking males during the low fruiting period. These findings suggest not only that males gain energetic benefits from high rank, but that rank might matter most not during unfavorable feeding periods, when individuals generally resort to fallback foods, but during the more salubrious times when party size increases and priority of access to high quality resources could affect the amount of surplus energy obtained. Although expanded analyses will indicate whether these preliminary results represent a robust pattern, Pusey et al. (2005) found that high ranking male chimpanzees at Gombe showed reduced fluctuations in body weight and, therefore, may be more energetically buffered than low ranking males.

In sum, the results from the wild chimpanzee study demonstrated that relatively high urinary C-peptide levels were associated with the well-fed conditions of captivity as well as favorable feeding conditions and high social status for males in the wild.

**Wild orangutan study**

Urinary C-peptide values for the wild orangutan samples ranged from below detection to 16.2 ng/mg Cr/ml. A dilution factor of 1:3 provided the most concentrated urine specimen possible from the reconstituted filter paper samples with sufficient volume to measure C-peptide in duplicate as well as creatinine. Although sample size was small, we noticed a clear trend. None of the urine samples from the low fruiting period, two of which contained ketones, registered a detectable urinary C-peptide level. Conversely, all the samples collected during the mast fruiting episode showed detectable levels of urinary C-peptide. These data are summarized in Table 1. The highest urinary C-peptide value (16.2 ng/mg Cr/ml) corresponded to the peak in the mast fruiting event. The two remaining sample values were substantially lower and coincided with the onset of the mast (1.6 ng/mg Cr/ml) and shortly after the mast had subsided (2.1 ng/mg Cr/ml).

These preliminary findings suggest that orangutans may exhibit more pronounced fluctuations in urinary C-peptide level than chimpanzees in the wild. Since in-

---

**Fig. 3.** Comparison of mean (with standard error) baseline urinary C-peptide levels from captive and wild chimpanzees.

**Fig. 4.** Comparison of mean (with standard error) baseline urinary C-peptide levels for the two highest-ranking and two lowest-ranking male chimpanzees during the high fruiting period.
insulin operates in humans to either promote or prohibit energy storage. C-peptide values in orangutans could conceivably reach maximum levels during periods of mast fruit abundance and minimum levels during periods of low fruit availability. When orangutans break down fat reserves to the extent that ketones appear in the urine, urinary C-peptide may reach such extremely low levels as to be beyond detection. Finding out whether these patterns hold for orangutans in the wild would improve our understanding of urinary C-peptide as an energetic signal in this species.

**DISCUSSION AND CONCLUSION**

We successfully measured C-peptide in urine samples from wild chimpanzees and orangutans and showed that baseline urinary C-peptide level provides a physiological marker of energetic status in these species. Certain issues remain to be resolved, however, to utilize urinary C-peptide as an energetic signal for hypothesis testing. In the discussion that follows, we outline steps for continued development of the C-peptide technique and highlight promising research applications.

**Method development**

In our estimation, further refinement of the C-peptide method should include three primary tasks. First, our initial finding that urinary C-peptide serves as an energetic signal in chimpanzees and orangutans needs to be validated with a larger sample size of animals. Increased sample size extended over a longer study period would verify whether urinary C-peptide levels fluctuate reliably with variable feeding conditions. Second, it would be useful to verify whether the magnitude of the C-peptide signal is greater for orangutans than chimpanzees in the wild, since our preliminary results suggest that seasonal or cyclic fluctuations in baseline insulin production may be more extreme for orangutans than chimpanzees.

Third, the range of natural variation in urinary C-peptide levels needs to be determined for each species in the wild, including the extent of variation both within and between individuals under variable feeding conditions. It would be important also to examine the degree of daily fluctuation in C-peptide levels to determine how much noise might be present in the signal from day to day. Further knowledge of naturally occurring variation will make it possible to integrate sample values appropriately for data analyses, define meaningful variables for hypothesis testing, and design sound sampling strategies for the future.

Continued method development along the lines outlined above would not only complete the validation process but also generate new data on the metabolic physiology of great apes in the wild, an under-explored research area.

**Potential research applications**

One promising application of the urinary C-peptide technique involves studying the energetics of great ape reproduction in the wild, especially the regulation of female fecundity. Insulin has been shown in humans to influence ovarian hormone production (Poretsky and Kalin, 1987; Stamatakis et al., 1996). A significant association between urinary C-peptide and ovarian hormone levels in great apes would support the premise that insulin serves as a physiological link between feeding ecology, energetic condition, and female reproductive function (Ellison, 2001; Sherry, 2002). Urinary C-peptide could also be used to quantify the energetic costs of lactation, an aspect of great ape reproduction that has been difficult to study in the past.

In addition to research on great apes, urinary C-peptide could be used for more fine-grained analyses of energetic status in human forager populations (Sherry and Marlowe, 2007; Draper and Howell, 2005) and to frame the results in comparative context. Without comparative data sets, for example, it remains difficult to assess whether prominent energetic buffering mechanisms in hunter-gatherer societies, such as food sharing and sexual division of labor (Winterhalder, 1986; Lancaster and Lancaster, 1987), signify a major reduction in variance for humans relative to great apes. In addition, given the high incidence of obesity and diabetes among modern human societies, the study of insulin/glucose dynamics in forager populations would improve our understanding of the evolutionary parameters of human metabolic physiology with important implications for health and disease (McGarvey et al., 1989; Lieberman, 2003).

**ACKNOWLEDGMENTS**

This research was made possible by the contributions and support of many valued colleagues. We thank Richard Wrangham, Martin Muller, and Melissa Emery Thompson for the urine samples from wild chimpanzees, Cheryl Knott for the urine samples from wild orangutans, Stephanie Fíros Anestis for the urine samples from captive chimpanzees at New Iberia Research Center, and Judith Flynn Chapman for the urine samples from humans. Yerkes Regional Primate Center provided the matched serum and urine samples from captive chimpanzees. We thank Susan Lipson for technical assistance and Benjamin Campbell and Diagnostic Systems Laboratories, Inc. for assistance with laboratory costs. For helpful comments and conversations, we thank everyone above along with Richard Bribiescas, Kathryn Clancy, Ian Gilby, Tara Harris, Sonya Kahlenberg, Frank Marlowe, Andrew Marshall, Mary O’Rourke, David Pilbeam, and Rebecca Stumpf. We also wish to thank Joseph Kemnitz for setting us on the path of C-peptide in the first place. Suggestions from Donna Holmes Parks, Grażyna Jasienska, and two anonymous reviewers greatly improved an earlier version of the manuscript.

**LITERATURE CITED**


---

**TABLE 1. Urinary C-peptide values (ng/mg Cr/ml) for three wild orangutans during contrasting fruiting periods**

<table>
<thead>
<tr>
<th>Individual</th>
<th>Fruiting period</th>
<th>Male</th>
<th>Female Peak</th>
<th>Female Onset</th>
<th>Female Onset, 1.6 ND</th>
<th>ND</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Mast, 1.6</td>
<td>ND</td>
<td>16.2</td>
<td>1.6</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>ND</td>
<td>2.1</td>
<td></td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not detected.

* Sample contained ketones.


