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RESEARCH ARTICLE

Hyperprogesteronemia in Response to *Vitex fischeri* Consumption in Wild Chimpanzees (*Pan troglodytes schweinfurthii*)

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Chimpanzees in Gombe National Park consume fruits of *Vitex fischeri* during a short annual fruiting season. This fruit species is a member of a genus widely studied for phytoestrogen composition and varied physiological effects. One particularly well-studied species, *V. agnus-castus*, is noted for its documented effects on female reproductive function, evidenced in increased progesterone levels and consequent regulation of luteal function. We examined reproductive hormone levels in both male and female chimpanzees during a 6-week period of intense *V. fischeri* consumption. *V. fischeri* consumption was associated with an abrupt and dramatic increase in urinary progesterone levels of female chimpanzees to levels far exceeding the normal range of variation. Female estrogen levels were not significantly impacted, nor were male testosterone levels. These are some of the first data indicating that phytochemicals in the natural diet of a primate can have significant impacts on the endocrine system, though the fluctuating nature of chimpanzee diet and reproductive function does not allow us to determine whether the effects observed during this short period had a broader positive or negative impact on female fertility. Given the widespread use of various *Vitex* species by African primates and the as-yet-undescribed phytochemical properties of these species, we predict that our observations may be indicative of a broader phenomenon. Am. J. Primatol. 70:1064–1071, 2008.

Key words: diet; phytoestrogens; reproduction; progesterone; chimpanzees

INTRODUCTION

There is a robust literature on the potential for phytoestrogens and other chemical constituents of natural plant foods to influence the reproductive physiology of humans and other animals. Phytoestrogens, chemicals having structural and/or functional similarities to endogenous estrogens, can influence the reproductive system via several pathways, including steroid receptor densities and affinities, cell signaling pathways, cell proliferation, and steroid biosynthesis [Whitten & Naftolin, 1998; Whitten & Patisaul, 2001]. Specific reproductive effects have been demonstrated from administered diets rich in phytoestrogens, though the nature of these effects vary and may be positive or negative depending on the specific compounds administered, doses, and the timing and the duration of administration [review: Whitten & Patisaul, 2001]. Naturally phytoestrogen-rich diets have a variety of inhibitory effects on reproduction in herbivores [Adams, 1995; Hughes, 1988] and quail [Leopold et al., 1976]. Evidence for phytochemical influences on the reproductive physiology of primates is primarily limited to laboratory studies [Foth & Cline, 1998; Harrison et al., 1999; Hopkins et al., 1977; Trisomboon et al., 2004; Wood et al., 2006]. However, Whitten [1983] noted a close correlation of seasonal mating behavior in wild vervet monkeys (*Cercopithecus aethiops*) with the consumption of estrogenic *Acacia elatior* flowers [Garey et al., 1992], and other researchers have speculated that the conspicuous consumption of particular foods rich in phytochemicals may alter the reproductive function of their study animals [Strier, 1993; Wallis, 1997]. Recently-published research documents elevated progesterone in response to consumption of *Vitex doniana* fruits by baboons in Gashaka-Gumti National Park, Nigeria

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(Higham et al., 2007). In this study, we provide strong evidence for elevation of progesterone levels in female chimpanzees as the direct result of the consumption of the fruit *Vitex fischeri*.

Researchers have described a wide range of biological effects of phytoestrogens and other compounds contained in several members of the plant genus *Vitex* [e.g., *V. negundo*: Bhargava, 1989; *V. doniana*: Eckman & Hines, 1993; *V. polygama*: Goncalves et al., 2001; *V. trifolia*: Li et al., 2005; *V. rotundifolia*: Okuyama et al., 1998; *V. altissima*: Sridhar et al., 2005; *V. peduncularis*: Suksamrarn et al., 2002]. These plants and their constituents have variously been cited for their effectiveness as antiinflammatory [e.g., Chawla et al., 1992], antibacterial [Sridhar et al., 2005], antidiarrheal [Agunu et al., 2005], antihistamine [Alam et al., 2002], analgesic [Dharmasiri et al., 2003], antiviral [Goncalves et al., 2001], antioxidant [Gallo et al., 2006], antitrypanosomal [Kiuchi et al., 2004], antifungal [Hernandez et al., 1999], anticonvulsant [Tandon & Gupta, 2005], cytotoxic [Hirobe et al., 1997], antiandrogenic [Das et al., 2004], insecticidal [Pushpalatha & Muthukrishnan, 1995], antivenin [Alam & Gomes, 2003], and profertility agents [Eckman & Hines, 1993]. The most widely cited effects are those of *V. agnus-castus* (VAC), the fruits of which are cited in both historical and modern usage for their impact on the reproductive physiology of one or both sexes [Daniele et al., 2005]. Fruit extracts of VAC induce increases in female progesterone levels and are widely employed as natural remedies for premenstrual syndrome and menstrual irregularities [Girman et al., 2002; Tesch, 2001; Wuttke et al., 2003]. The German government has approved its use for treatment of premenstrual syndrome, breast pain, and irregular menstrual cycles [Hardy, 2000]. Double-blind studies in that country found that *Vitex* extract was effective in treatment of luteal deficits [Gerhard et al., 1998; Milewicz et al., 1993] and premenstrual syndrome [Schellenberg, 2001], presumably as a result of its effects on progesterone levels. VAC's effects on male reproductive physiology are suggested by its traditional use among monks to reduce libido (hence the common name "chasteberry"), but clinical data are lacking.

Chimpanzees in the Gombe National Park in Tanzania naturally consume the fruits of a related species, *V. fischeri,* during its short annual fruiting season [Goodall, 1968]. The phytochemistry of this *Vitex* species has not yet been described. Here, we test for potential effects of *V. fischeri* consumption on reproductive hormones in both male and female chimpanzees at Gombe.

METHODS

Study Location Community

The Kasekela community of chimpanzees in Gombe National Park, Tanzania, has been the subject of continuous research since 1960 [Goodall, 1986]. The park, spanning 35 km^2 along the shores of Lake Tanganyika, is composed of a mosaic of grassland and semideciduous and evergreen forests extending from elevations of 770–1,600 m [Goodall, 1986; Wrangham, 1977; Pussey et al., 2008]. In the beginning of 2002, the Kasekela community contained 51 individuals, including 20 adult and subadult females and 12 adult and subadult males. Research with Gombe chimpanzees was conducted in compliance with the Institutional Animal Care and Use Committees at Harvard University and the University of Minnesota and with permission from Tanzanian governmental authorities.

Behavioral Sampling

Field researchers at Gombe conduct daily nest-to-nest observations of Kasekela chimpanzees. If possible, one focal individual is followed throughout the day; feeding and social behavior of the focal are recorded continuously and party membership is recorded every 15 min. The plant part and species of all items eaten are recorded. A Tanzanian botanist (G. G.) maintains an on-site herbarium collection and helps with identification of plant species. We calculated dietary composition based on the items consumed by focal animal at the time of each 15-min scan sample. Note that the dates of the *Vitex* season were defined by the first and last observations of any focal animal consuming the fruit and that observers traveled with focal animals rather than observing *Vitex* trees full time. Our field data do not permit us to calculate the dates and intensity of *Vitex* feeding by individual chimpanzees.

Endocrine Sampling and Laboratory Analysis

Between June 2001 and December 2002, we collected fresh, uncontaminated samples by placing a plastic sheet underneath a known subject or by pipetting from vegetation. Samples were frozen as quickly as possible, within a time frame (0–14 hr) that has been demonstrated not to significantly affect ovarian hormone or creatinine concentrations [Kesner et al., 1995].

M. E. T. assayed urine samples in the Primate Reproductive Ecology Laboratory at Harvard University using competitive enzyme-immunoassay (reagents c/o, C. J. Munro, University of California, Davis). Female urine samples were assayed for the urinary metabolites of estrogen (estrone conjugates, E_1C) and progesterone (pregnanediol-3-glucuronide, PdG). We assayed male samples for testosterone after conducting a hydrolysis and extraction procedure to deconjugate the key urinary metabolite, testosterone glucuronide [Mohle et al., 2002; Muller & Wrangham, 2004]. Enzyme-immunoassay procedures followed previously published protocols [e.g., Czekala et al., 1986; Emery Thompson, 2005a; Shideler et al., 1990] with coating dilutions for Nunc Maxisorp immunoplates at 1:6,000 for estrone-β-glucuronide (Antibody

R522-2), 1:30,000 for PdG (Pab 13904), and 1:10,000 for testosterone (R156-7). We standardized all results for creatinine (Cr) concentration to adjust for sample concentration [Taussky, 1954]. Interassay coefficients of variation (CVs) were 13.6% for E_1C ($n = 56$), 14.2% for PdG ($n = 26$), and 11.5% for testosterone ($n = 17$). Intraassay CVs from 6 replicate urine samples were 4.9% for E_1C, 3.4% for PdG, and 5.6% for testosterone.

Data Analysis

For males, we considered morning (before 10 am) and "afternoon" (after 10 am) samples separately owing to circadian effects on urinary testosterone concentrations in chimpanzees [Muller & Lipson, 2003]. We included both adult and subadult (>10 years of age) males. For females, we categorized each urine sample according to the reproductive state of the subject based on birth records, sexual swelling occurrence, and pregnancy testing. In order to obtain a valid statistical comparison of hormonal levels in females of the same reproductive status before, during, and after *Vitex* season, we limited our analysis primarily to noncycling females. This included adolescent females at least 8 years of age and females in the period of lactational amenorrhea before resumption of cycling. To test for significant changes in hormonal values, we used Wilcoxon matched-pairs tests ($\alpha = 0.05$, two-tailed significance) to compare individual chimpanzees' average values over the 6-month period before the first observation of *Vitex* consumption with their average values during *Vitex* consumption (first to last observation: 17 February–26 March 2002). We also compared paired values from the period of *Vitex* consumption and average levels in the 6 months after the last *Vitex* feeding observation.

RESULTS

Kasekela chimpanzees were observed consuming *V. fischeri* fruits (Fig. 1) between 17 February 2002 and 26 March 2002. During this time period, 1,547 scan observations were recorded (approximately 387 observation hours), with focal animals feeding for 797 (51.5%) of these observation points. Fruits of *V. fischeri* were the most common dietary item for the chimpanzees over this period, accounting for 17.3% of feeding observations. Seventeen of 25 known Kasekela females and 10 of 12 known males were observed in feeding parties at *V. fischeri* trees.

We compared mean urinary reproductive hormone concentrations in male and female chimpanzees during the *Vitex* season with mean concentrations from the same individuals during the 6 months preceding and the 6 months following the season. Urinary progestin levels of Gombe females were significantly elevated in the *Vitex* season compared with the previous 6-month period (Wilcoxon signed-ranks test, $z = -2.023$, $N = 5$ females, $P = 0.043$) and the subsequent 6-month period ($z = -2.521$, $N = 8$, $P = 0.012$, Fig. 2a). Elevated values were tightly tied to the dates of our *Vitex* feeding observations and fell outside of the typical range of variation for PdG (Fig. 3). PdG levels of noncycling females during the *Vitex* season averaged $4{,}130 \pm 923$ ng/mg-Cr (mean $\pm$ standard error, $N = 5$ females), more than four standard deviations above their typical levels (810 ± 232 ng/mg-Cr, $N = 14$ females sampled from June 2001 to December 2002). We were only able to collect seven samples from four cycling females during the *Vitex* season; all were collected during the presumed follicular phase [i.e., during maximal swelling or 10 days earlier; Emery Thompson, 2005a], a time of characteristically low progesterone activity. These samples averaged $9{,}121 \pm 4{,}519$ ng/mg-Cr, three standard deviations above their usual levels ($1{,}964 \pm 765$ ng/mg-Cr, $N = 10$ females).

Fig. 1. Photos of *Vitex fischeri* from Gombe National Park, illustrating (**a**) ripe (black) and unripe (green) fruits and (**b**) mature leaves with fruits (photos: Michael Wilson).

In contrast, urinary estrogen levels of females during the *Vitex* season were not significantly different from values during the previous 6 months ($z = -1.214$, $N = 5$, $P = 0.225$), which were slightly higher on average, and values during the following 6 months ($z = -1.680$, $N = 8$, $P = 0.093$), which were slightly lower (Fig. 2b).

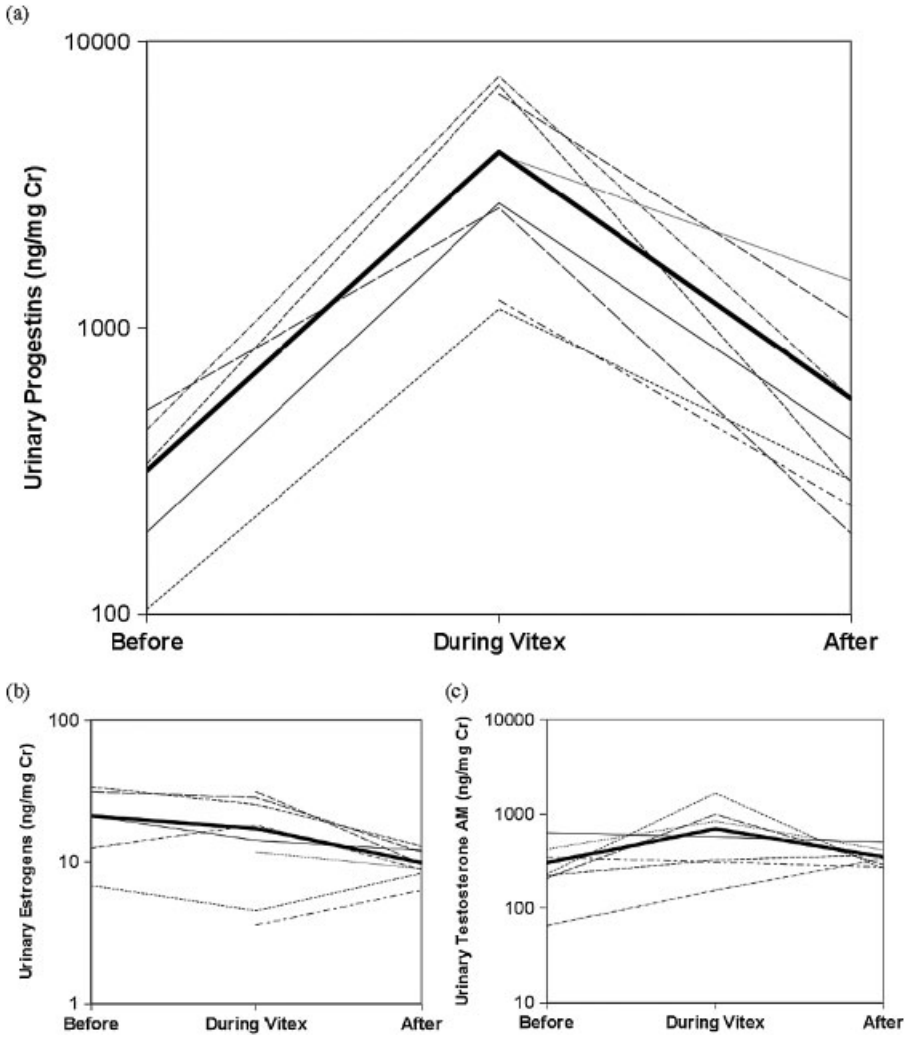

Fig. 2. Urinary hormone measurements of chimpanzees during the *Vitex* season (17 February 02–26 March 02) compared with the 6 months before and 6 months after. Each thin solid or dotted line indicates an individual chimpanzee, whereas the solid thicker line denotes the mean across individuals. *Y*-axes use a log-scale to accommodate large effects. (**a**) Urinary PdG levels of noncycling females; (**b**) urinary E_1C levels of noncycling females; and (**c**) urinary testosterone of males in morning samples. PdG, pregnanediol-3-glucuronide; E_1C, estrone conjugates.

Male urinary testosterone levels were also not significantly affected by *Vitex* feeding. Neither morning nor afternoon testosterone levels differed significantly from the previous 6 months (morning: $z = -1.859$, $N = 7$ males, $P = 0.063$; afternoon: $z = -1.352$, $N = 6$, $P = 0.176$) or the following 6 months (morning: $z = -0.944$, $N = 7$, $P = 0.345$; afternoon: $z = -0.280$, $N = 9$, $P = 0.779$, Fig. 2b). Testosterone levels were slightly elevated on average, which contrasts with the prediction based on VAC's purported impact on male libido.

DISCUSSION

The consumption of *V. fischeri* fruits coincided with an abrupt and dramatic elevation in progesterone levels of female chimpanzees at Gombe. This effect parallels the increase in progesterone observed from the consumption of VAC extracts by human females. Precise causes of these effects are not known, but at least in the case of VAC do not appear to be related directly to a progesterone-like compound in the plant. Extracts of VAC fruits increase progesterone production in women via upregulation of progesterone receptors and modulation of the follicle-stimulating hormone/luteinizing hormone ratio [Girman et al., 2002; Liu et al., 2001; Tesch, 2001]. Reproductive hormones may also be altered through VAC's dopaminergic and antiprolactinemic effects [Meier et al., 2000; Sliutz et al., 1993; Tesch, 2001; Wuttke et al., 2003] and via competitive binding to estrogen α and β receptors [Liu et al., 2001, 2004].

The described effects of VAC consumption are primarily beneficial for fecundity in women [Girman et al., 2002; Milewicz et al., 1993; Schellenberg, 2001; Tesch, 2001; Wuttke et al., 2003]. This contrasts with

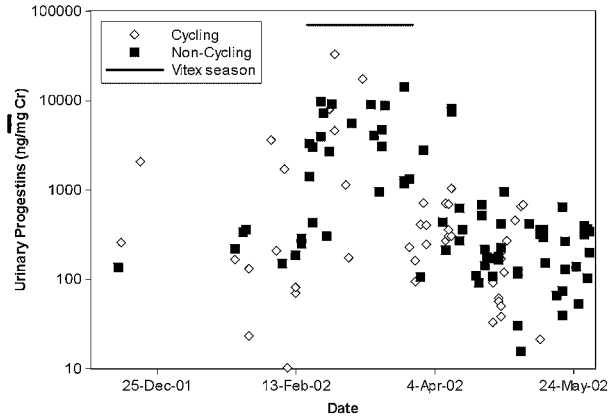

Fig. 3. Scatterplot of the urinary PdG concentrations of individual samples collected from cycling (open diamond) and noncycling (closed square) females, illustrating the close relationship between *Vitex fischeri* consumption and dramatically increased progestin levels. The period of *V. fischeri* feeding observations is noted by a solid black line. PdG, pregnanediol-3-glucuronide.

the hypothesized effects of many phytoestrogen compounds in plants, as well as the observed negative effects of such compounds on reproduction in herbivores and other species [Adams, 1995; Hughes, 1988; Leopold et al., 1976; Trisomboon et al., 2004]. Although many phytoestrogens appear to have positive effects on health, particularly on prevention of cancers and cardiovascular disease [Ososki & Kennelly, 2003], their effects on reproductive health and fertility are frequently negative or inhibitory [Jefferson & Newbold, 2000; Whitten & Patisaul, 2001]. In our study, estrogen levels of chimpanzees were not significantly outside of the normal range of variation during *V. fischeri* consumption. We do not know whether the observed effects on progesterone levels in chimpanzees affected fecundity, positively or negatively. The occurrence of sexual swellings was relatively high but not unusual, and no conceptions occurred during the *Vitex* season or during the following 6 months. Given long interbirth intervals and the typical seasonal variation in chimpanzee reproductive function [Anderson et al., 2006; Emery Thompson, 2005a; Emery Thompson & Wrangham, 2008; Wallis, 1995, 2002], the potential effect of *Vitex* consumption on fecundity is difficult to evaluate. However, *Vitex* has been used annually by Gombe chimpanzees since at least 1974 and the feeding season is often longer or more intense than it was in 2002 [Gombe Stream Research Centre, unpublished data]. Such habitual use would seem to be disfavored were the fruit to produce strong negative fertility effects. However, it is also possible that the duration of altered hormonal function during any given *Vitex* season is insufficient to produce significant disruptions or enhancements in female fertility.

Reproductive hormones may by influenced by the diet through other pathways. In chimpanzees, as in humans [Ellison et al., 1993] and orangutans [Knott, 2001], ovarian hormone levels correlate with increased consumption of foods high in nutritional quality [Emery Thompson & Wrangham, 2008; Emery Thompson et al., 2007]. However, this is unlikely to be the cause of the hormonal effects we found because overall ripe fruit consumption during February and March 2002 was relatively low. Furthermore, in chimpanzees (including those at Gombe), the acute effects of increased dietary quality are seen primarily in increasing estrogen levels, whereas progesterone responses are often dampened or delayed [Emery Thompson, 2005b; Emery Thompson & Wrangham, 2008]. The responses observed here also far exceed those observed during seasonal shifts in food availability for chimpanzees.

VAC consumption has historically been linked with reduced male sexual motivation and has been used by monks for this purpose [Daniele et al., 2005]. However, we are not aware of any clinical evidence to suggest effects on testicular function in humans. In our study of *V. fischeri* consumption, we did not detect significant changes in the testosterone levels of male chimpanzees.

Vitex species feature prominently in the diets of chimpanzees [*V.* sp.: Azuma & Toyoshima, 1961; *V. doniana*: Duvall, 2000; Moscovice et al., 2007; Sugiyama & Koman, 1987; Tutin & Fernandez, 1993; *V. madiensis*: McGrew et al., 1988; *V. grandifolia:* Poulsen et al., 2001; *V. cienkowski*: Sugiyama & Koman, 1987; *V. ferruginea*: Suzuki, 1969], bonobos [*V.* sp.: Badrian et al., 1981], gorillas [*V.* sp.: Cousins & Huffman, 2002; Rogers et al., 2004; *V. doniana*: Nishihara, 1995; Rogers et al., 1990; Williamson et al., 1990; *V. grandifolia*: Poulsen et al., 2001], and other primates [*V. grandifolia*: Poulsen et al., 2001] in sites across Africa. Like *V. fischeri*, the chemical compositions of these *Vitex* species are as yet undescribed. There is therefore ample potential to study whether phytochemical influences of *Vitex* on female hormone levels are a more general phenomenon, and whether broader reproductive effects result from it, particularly where usage is more intense. Such further evidence would raise the intriguing possibility that primates select these plants for their positive phytochemical rather than nutritional properties, just as they consume plants with particular mechanical or chemical properties for apparent medicinal purposes [Carrai et al., 2003; Huffman, 1997].

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