

Thyroid hormone concentrations in female baboons: Metabolic consequences of living in a highly seasonal environment

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ABSTRACT

How female mammals adapt metabolically in response to environmental variation remains understudied in the wild, because direct measures of metabolic activity are difficult to obtain in wild populations. However, recent advances in the non-invasive measurement of fecal thyroid hormones, triiodothyronine (T3), an important regulator of metabolism, provide an opportunity to understand how female baboons living in the harsh Amboseli ecosystem in southern Kenya adapt to environmental variability and escape strict reproductive seasonality. Specifically, we assessed how a female's activity budget, diet, and concentrations of fecal T3 metabolites (mT3) changed over the course of the year and between years. We then tested which of several environmental variables (season, rainfall, and temperature) and behavioral variables (female activity budget and diet) best predicted mT3 concentrations. Finally, we determined if two important reproductive events – onset of ovarian cycling and conception of an offspring – were preceded by changes in female mT3 concentrations. We found female baboons' mT3 concentrations varied markedly across the year and between years as a function of environmental conditions. Further, changes in a female's behavior and diet only partially mediated the metabolic response to the environment. Finally, mT3 concentrations increased in the weeks prior to menarche and cycling resumption, regardless of the month or season in which cycling started. This pattern indicates that metabolic activation may be an indicator of reproductive readiness in female baboons as their energy balance is restored.

1. Introduction

Animals living in the wild are exposed to seasonal changes in rainfall, temperature, and food. Many species have adapted to this variation in resources by reproducing seasonally, allowing them to conceive and/or produce offspring when environmental conditions are favorable (Bronson, 1985). However, seasonal reproduction is not always an advantageous strategy. For example, strictly seasonal reproduction may lead to missed reproductive opportunities for species with long reproductive cycles that last more than a year, and in unpredictable environments,

where rainfall and food abundance are highly variable from year to year (Heldstab et al., 2021). Several environmental variables – including high mean annual temperatures and tropical latitudes – are associated with an escape from strictly seasonal reproduction (reviewed in Heldstab et al., 2021).

For species that do not follow strict seasonal reproduction, the timing of reproduction is not completely governed by external environmental cues (e.g., temperature, day length), but relies primarily on internal cues such as an animal's energetic condition (Janson and Verdolin, 2005; Heldstab et al., 2021). Energetic condition depends on the amount of

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energy an animal has stored as well as its energy balance (i.e., the difference between its energy intake and energy expenditure; Ellison, 2001). Energy intake and expenditure, in turn, ultimately depend upon the availability of food, the demands of obtaining it, and the metabolic adaptations an animal employs as these variables change over time. To accommodate the high costs of reproduction during periods of food scarcity, non-seasonal breeders have evolved physiological and behavioral responses (e.g., reduction of metabolism, changes in activity and/or diet) to limit the energy deficit and ensure that they have enough energy to reproduce as well as perform essential functions (Alam et al., 2003; Butte and King, 2005; reviewed in Bronson, 1995; Heldstab et al., 2021).

Characterizing the metabolic adaptations that animals employ in response to changes in rainfall, food, and temperature is of key importance and remains understudied in wild populations where direct measures of metabolism have been difficult to obtain. However, recent advances in the non-invasive measurement of fecal thyroid hormones provide an opportunity to examine how metabolism is modulated in species that live in a highly seasonal environments and whether metabolic adaptations help maintain their energy balance (Wasser et al., 2010; Gobush et al., 2014; Cristobal-Azkarate et al., 2016; Dias et al., 2017; Gesquiere et al., 2018a).

Here, we report on a non-invasive analysis of fecal triiodothyronine metabolites (mT3) in the well-studied population of baboons (*Papio cynocephalus*/*P. anubis* admixture) in Amboseli, Kenya. We investigate how behavioral, dietary, and metabolic flexibility might help baboons to alleviate the energetic constraints of a semi-arid and unpredictable habitat and support non-seasonal breeding. Baboons, which are large, semi-terrestrial, non-seasonally breeding primates, show evidence that their reproduction is subject to energetic constraints. For example, female baboons are less likely to cycle and to conceive after periods of drought or extreme heat (Beehner et al., 2006a), and post-partum amenorrhea is prolonged (e.g., lactation is prolonged and resumption of cycling delayed) in periods of low rainfall when food is scarce (Packer et al., 1995; Gesquiere et al., 2018b). However, despite these energetic constraints, baboons in Amboseli show only weak reproductive seasonality: births occur in all months of the year but are most likely to occur between January and June (Alberts et al., 2005; Campos et al., 2017). This is in contrast, for instance, to vervet monkeys (*Cercopithecus aethiops*), terrestrial non-human primates in the same habitat, which forage on fewer food types and experience a strong seasonal pattern of reproduction (Struhsaker, 1967; Wrangham and Waterman, 1981; Janson and Verdolin, 2005). By examining variation in the concentration of mT3 in the fecal samples of female baboons we gain insight into how these animals flexibly adapt and breed year-round in the highly seasonal environments in which they live.

1.1. Thyroid hormones as indicators of metabolic demands

Thyroid hormones are secreted by the thyroid gland, which produces two main thyroid hormones in vertebrates: triiodothyronine (T3) and its prohormone, thyroxine (T4). Thyroid hormones stimulate glucose production and lipid metabolism and have key roles in the regulation of heat production and basal metabolic rate (BMR). When individuals are exposed to energetic demands (e.g., mating, pregnancy) or to low temperatures, their total (sum of free and bound) T3 concentrations increased and their metabolism is activated, leading to energy and heat production (Glinoe, 1997; Silva, 2003; Chatzitomaridis et al., 2017). However, when energy intake is limited (e.g., during food shortage, food restriction), T4 conversion into T3 is substantially reduced, and free and total T3 concentrations decrease. This process leads to a decrease in metabolic activity and heat production and allows individuals to conserve their energy reserves (Eales, 1988, see review by Chatzitomaridis et al., 2017). Because fecal T3 metabolites reflect circulating T3 concentrations, they have been used as an indicator of metabolic activity, with high concentrations indicating an activation of the metabolism and

low concentrations indicating a reduction of metabolic activity (Keech et al., 2010; Mondol et al., 2020; Chmurska-Gąsowska et al., 2021).

The role of T3 in responding to different types of energetic demands has been examined in many species by measuring mT3 concentrations in feces. Studies in captive howler monkeys (*Alouatta palliata*) and capuchin monkeys (*Sapajus xanthosternos*) have shown that fecal mT3 concentrations vary with food intake, and that food restriction leads to a decrease in mT3 concentrations (Wasser et al., 2010; Schaebis et al., 2016). Similar findings have been reported in several species in the wild, where low food availability (measured directly or indirectly via rainfall, foraging time, or *Normalized Difference Vegetation Index*) has been associated with low mT3 concentrations (in Barbary macaques, *Macaca sylvanus*: Cristobal-Azkarate et al., 2016; in mantled howler monkeys, *A. palliata*: Dias et al., 2017; in African elephants, *Loxodonta africana*: Szott et al., 2020). Ambient temperatures have also been associated with changes in mT3 concentrations in several species, with high mT3 concentrations observed at low temperatures and low mT3 concentrations observed at high temperatures (in impalas, *Aepyceros melampus*: Thompson et al., 2017; in Japanese macaques, *Macaca fuscata*: Hunnink et al., 2020; in African elephants, *L. africana*: Szott et al., 2020). However, in a number of species the effect of cold temperatures on mT3 concentrations appears to be modulated by the energy availability: animals exposed simultaneously to low temperature and food shortage (e.g. during winter), showed evidence of hypometabolism, low BMR, and low mT3 concentrations (in red deers, *Cervus elaphus*: Arnold et al., 2004; in Alpine ibex, *Capra ibex ibex*: Signer et al., 2011; in Shetland pony, *Equus caballus* L.: Brinkmann et al., 2014; in moose, *Alces alces*: Græsli et al., 2020; in muskox, *Ovibos moschatus*: Desforges et al., 2021).

The relationship between mT3 concentrations and female reproductive state has been variable across studies. For instance, in mantled howler monkeys (*A. palliata*), fecal mT3 concentrations were highest in pregnant females, intermediate in lactating females, and lowest in cycling females (Dias et al., 2017), while in Hawaiian monk seals (*Monachus schauinslandi*) fecal mT3 concentrations did not differ across reproductive states (Gobush et al., 2014). Finally, previous research in our study population showed that in baboons (*P. cynocephalus*/*P. anubis* admixture), mT3 concentrations were lowest in pregnant and post-partum amenorrhea females and highest in cycling females (Gesquiere et al., 2018a). These differences across studies may result of species-level differences in metabolic patterns, or they may be linked to variation in the amount of energy available to different populations within a species, as suggested by findings in humans (Prentice and Goldberg, 2000). Specifically, whether BMR increases or decreases during human pregnancy depends upon nutritional condition: in well-nourished women, an increase in BMR is observed during pregnancy, but in undernourished women a decrease in BMR is found in the first two trimesters of pregnancy with only a slight increase in the last trimester (Prentice and Goldberg, 2000). Because of the important role of T3 in regulating BMR, we can expect that mT3 concentrations, like BMR, will vary as a function of the species under study, and of the nutritional status of the animal, resulting in variation both within and between species relationship between mT3 concentrations and female reproductive state.

1.2. Goals of this study

We expand on previous work on predictors of mT3 concentrations in female baboons (Gesquiere et al., 2018a) by pursuing three objectives. First, we describe variation in environmental variables, baboon behavior, and mT3 concentrations. Specifically, we report considerable variation in mT3 concentrations, female activity budgets, and diets, across the hydrological year and between years, and we compare these measures to variation in rainfall and temperature.

Second, we ask which of the environmental and behavioral variables we examined (rainfall, temperature, season, female activity budget, and diet) best predict variation in mT3 concentrations, accounting for female reproductive state and group size. Because mT3 concentrations are

lower when food availability is low (Ayres et al., 2012; Cristobal-Azkarate et al., 2016; Thompson et al., 2017), we expect that variables associated with low food availability and/or low energy intake in Amboseli will be associated with low mT3 concentrations in female baboons. These include (i) the dry seasons when rainfall is reliably low, (ii) periods of low rainfall during any season (rains often fail even in the wet seasons), (iii) periods when time spent feeding is prolonged and time spent resting is reduced, and (iv) periods when the baboons' diet consists of a high percentage of corms and a low percentage of high-energy foods (see Table 1). Because thyroid hormones also have an important role in thermoregulation, and their concentrations are typically up-regulated when animals are exposed to cold temperatures and down-regulated when exposed to hot temperatures (Silva, 2003; Kahl et al., 2015; Thompson et al., 2017; Hunnink et al., 2020), we predict that mT3 concentrations in female baboons will be relatively high during colder weather and relatively low during hotter weather. We also predict that pregnant and post-partum amenorrhea females will have lower mT3 concentrations than cycling females as previously found in our species (Gesquiere et al., 2018a), and that mT3 concentrations will be lower in larger groups, as competition for food increase with group size (Altmann and Alberts, 2003; Markham and Gesquiere, 2017) (see Table 1).

Third, our previous work has suggested that female energy balance may be a key factor in determining the resumption of ovarian cycling after lactational amenorrhea, but not in determining the timing of conception or birth (Gesquiere et al., 2018b). We test these predictions with the aim of understanding whether the weakly seasonal timing of reproduction in Amboseli may be partially explained by variation in metabolic activity in the months prior to these reproductive events. Specifically, we predict that female baboons will be more likely to achieve menarche and to resume cycling post-partum during or following periods of elevated mT3 concentrations, but we do not expect to find a relationship between the timing of conceptions and mT3 concentrations.

2. Methods

2.1. Study population

Our study subjects were female baboons belonging to the well-studied population living in Amboseli (2°40'S, 37°15'E, 1100 m altitude), southern Kenya (Alberts and Altmann, 2012). Baboons are large semi-terrestrial monkeys that are widespread across sub-Saharan Africa (Jolly, 1993; Henzi and Barrett, 2003). Baboons have a diverse diet that encompasses grass, fruits, flowers, seeds, pods, and leaves from a variety of plants as well as tree gum, insects and meat on occasion (Norton et al., 1987; Whiten et al., 1991; Byrne et al., 1993; Altmann, 1998; Alberts et al., 2005; Altmann, 2009). Baboons selectively exploit foods as they become available, allowing them to achieve relative nutrient stability, even though not all foods consumed are equivalent in their nutrient content (Altmann et al., 1987; Byrne et al., 1993; Altmann, 1998). During the long dry season in Amboseli, when the availability of high-energy food sources (i.e., fruits, flowers, grass blades) is low, baboons rely heavily on grass corms (the underground storage organs of grasses). Although grass corms are reasonably rich in both protein and energy, they require considerable processing time, thus making their profitability low (Altmann et al., 1987; Byrne et al., 1993; Altmann, 1998). As a consequence, feeding time increases dramatically during the dry season largely at the expense of resting time (Alberts et al., 2005).

Baboons in Amboseli have been monitored on a near-daily basis since 1971 by the Amboseli Baboon Research Project (ABRP), including collection of reproductive, demographic, behavioral and hormonal data (e.g., Alberts and Altmann, 2012). The population consists of yellow baboons (*P. cynocephalus*) that experience some natural admixture with neighboring populations of olive baboons (*P. anubis*) (Alberts and Altmann, 2001; Tung et al., 2008; Charpentier et al., 2012). These two

species show little evidence of ecological differentiation (Winder, 2014; Wango et al., 2019) and produce viable and fertile offspring with little or no evidence of hybrid dysgenesis (Ackermann et al., 2006; Tung et al., 2008). Social groups in this population vary in size and change dynamically over time, and in the present study, group size ranged from 14 to 119 total individuals (mean \pm SD: 61 \pm 21).

All data collection procedures were non-invasive, adhered to the laws and guidelines of Kenya (Research Permit NACOSTI/P/22/22332), and were approved by the Animal Care and Use Committee at Duke University (IACUC A028-12-02).

2.2. Study subjects and datasets

We used hormonal and behavioral data collected between 2005 and 2018 from adult and immature females. For objectives 1 and 2, we focused on adult females, while for objective 3, we also included immature females for the analyses of menarche. For objectives 1 and 2, our dataset included 220 adult females for whom we had a total of 7107 fecal samples (mean \pm SD: 32 \pm 28 samples per female, range 1–129). For objective 3, we limited our dataset to the 6-month periods that preceded each of the three reproductive events most likely to be energy-dependent: (i) menarche (131 immature females for whom we had 378 fecal samples, with an average of 2.88 samples per female), (ii) cycling resumption (149 adult females for whom we had 1623 fecal samples, with an average of 10.9 samples per female), and (iii) conception (183 adult females for whom we had 1229 fecal samples, with an average of 6.72 samples per female).

2.3. Data collection and compilation for rainfall, temperature, and season

Our first and second objectives both required data on seasonal and annual variation in climate (rainfall and temperature). Amboseli lies in the region of Equatorial East African that is considered to have two rainy seasons and two dry seasons each year. Canonically, the “short rains” begin at the end of October and end in early December, and the “long rains” fall from March through May (Western and Maitumo, 2004; Camberlin et al., 2009; Lea et al., 2015; Philippon et al., 2015). Hence, we define the hydrological year as starting in November with the onset of the short rains and ending at the end of the long dry season at the end of October (Altmann et al., 2002; Alberts et al., 2005). Importantly, both the short and long rains exhibit high inter-annual variation in rainfall in Amboseli (Fig. 1a). In addition, while we define the four seasons according to average dates of onset and cessation of rainfall (as is traditional for East Africa), the ‘season’ variable in our model also captures variation in annual plant phenology – and therefore in nutritional content of baboon foods – that is not fully accounted for by variation in total rainfall alone. We discuss our modeling approach to address this variation within and between seasons below (see also Altmann et al., 2002; Alberts et al., 2005; Lea et al., 2015; Philippon et al., 2015).

2.3.1. Measuring rainfall and temperature

Daily records of rainfall were obtained using a rain gauge at the research field camp, located within 2–17 km of the ranges of the baboon study groups (Altmann et al., 2002). Daily values were summed to calculate monthly and annual rainfall for each hydrological year (November–October). Daily records of minimum and maximum temperature (T_{\min} and T_{\max} , respectively) were obtained using a shaded min-max thermometer at the research field camp. Average daily temperature (hereafter T_{av}) was calculated as the average of T_{\min} and T_{\max} on a given day. In our analyses, we used T_{av} instead of mean T_{\min} and T_{\max} because T_{\min} and T_{\max} were highly correlated (generalized variance inflation factors >5; Fox and Weisberg, 2019).

2.3.2. Defining seasons

We included season as well as rainfall and temperature in our models for two reasons. First, as stated above, the ‘season’ variable captures

Table 1

Variables used as predictors of mT3 concentrations in fecal samples. For statistical modeling, mT3 values represent the average of all females in a given reproductive condition in a group, within a season and year.

	Predictor	Predictor Description	Predictor type	Predicted effect	Prediction supported?
Environmental variables	Season	The season in which the fecal sample was collected. Seasons were: 'short rain' from October 27th through January 31st, the 'short dry' from February 1st through February 28-29th, 'long rain' from March 1st through May 15th, and the 'long dry' from May 16th through Oct 26th.	Fixed effect; categorical	mT3 concentrations will be lowest in the two dry seasons, because overall food quantity and quality should be poorest in these reliably dry seasons.	Yes (Table 3, Fig. 1c).
	Mean daily rainfall	Mean daily rainfall in the 30-days period prior to fecal sample collection.	Fixed effect; continuous	mT3 concentrations will be low during periods of low rainfall in any season, because food quantity and quality will generally be poorer when rainfall is lower.	Yes (Table 3, Fig. 1c).
	Mean daily average temperature	Mean daily average temperature (T_{av} , which is calculated as the average of T_{min} and T_{max} each day) in the 30-days prior to fecal sample collection.	Fixed effect; continuous quadratic term (across season) or linear (within season)	mT3 concentrations will be high when T_{av} is low, and low when T_{av} is high, because T3 secretion is thermogenic (produces heat).	No: mT3 is highest at intermediate values of T_{av} , and is low when T_{av} is hottest or coolest (Table 3).
Activities	% time feeding	For each fecal sample, the percent of time spent feeding by all the females of a given social group that are in the same reproductive state, calculated for each season and hydrological year.	Fixed effect; continuous	mT3 concentrations will be lowest when females spend the highest % time feeding because high % time feeding will generally be associated with eating lower-quality foods.	No: mT3 shows no relationship to % time feeding (estimate is near zero; Table 3, see also Table 2).
	% time resting	For each fecal sample, the percent of time spent resting for all the females of a given social group that are in the same reproductive state, calculated for each season and hydrological year.	Fixed effect; continuous	mT3 concentrations will be lowest when females spend the lowest % time resting because these periods will be associated with eating lower-quality foods and expending more energy to be active.	Yes (Table 3).
Food eaten	% corms eaten	For each fecal sample, the percent of females' feeding time devoted to grass corms by all the females of a given social group that are in the same reproductive state, calculated for each season and hydrological year.	Fixed effect; continuous	mT3 concentrations will be lowest when females spend the highest % of feeding time on grass corms, because grass corms are low-quality foods.	Yes (Table 3).
	% high-energy-foods eaten (HEF)	For each fecal sample, the percent of females' feeding time devoted to foods with high-energy value (grass blades, fruits, flowers, gum, and invertebrates) by all the females of a given social group that are in the same reproductive state, calculated for each season and hydrological year.	Fixed effect; continuous	mT3 concentrations will be lowest when females spend the least time eating HEF.	No: mT3 shows no relationship to % HEF (estimate is near zero; Table 3, see also Table 2).
Female and group traits	Reproductive state	The reproductive state that the female was experiencing at the time each fecal sample was collected from her: cycling (C), pregnant (P), or in post-partum amenorrhea (PPA).	Fixed effect; categorical	mT3 concentrations will be lower during PPA and pregnancy than during cycling (Gesquiere et al., 2018a).	Yes (Table 3).
	Group size	The total number of baboons in a given group on the day the fecal sample was collected	Fixed effect; continuous	mT3 concentrations will be lowest in the largest group, because intragroup competition for food increases with large group size.	No: mT3 concentrations change in the predicted direction but confidence intervals overlap zero (Table 3, see also Table 2).
	Storage	The number of months a fecal sample was stored from the date it was collected until it was assayed for mT3.	Fixed effect; continuous	No statistically significant change in mT3 predicted as a function of storage time (Gesquiere et al., 2018a).	Yes, confidence intervals for the estimate overlap zero (Table 3). However, storage appears in all models with $\Delta AIC_c < 2$, so it remains important to control for this variable in analyses of mT3.
Random effects	Hydrological year	Hydrological year in which the fecal sample was collected; see methods.	Random effect; categorical	mT3 will show variation associated with year-to-year variation in food availability that arises from variation in rainfall, temperature and plant phenology.	Yes (Fig. S5).
	Female ID	Identity of the female each fecal sample was collected from.	Random effect; categorical	mT3 will show individual-level variation among females.	Yes (Fig. S5).
	Group ID	Identity of the social group each female belonged to at the time each fecal sample was collected from her.	Random effect; categorical	mT3 will show group-level variation associated with differences between groups in the distribution and availability of different plant foods.	Yes (Fig. S5).

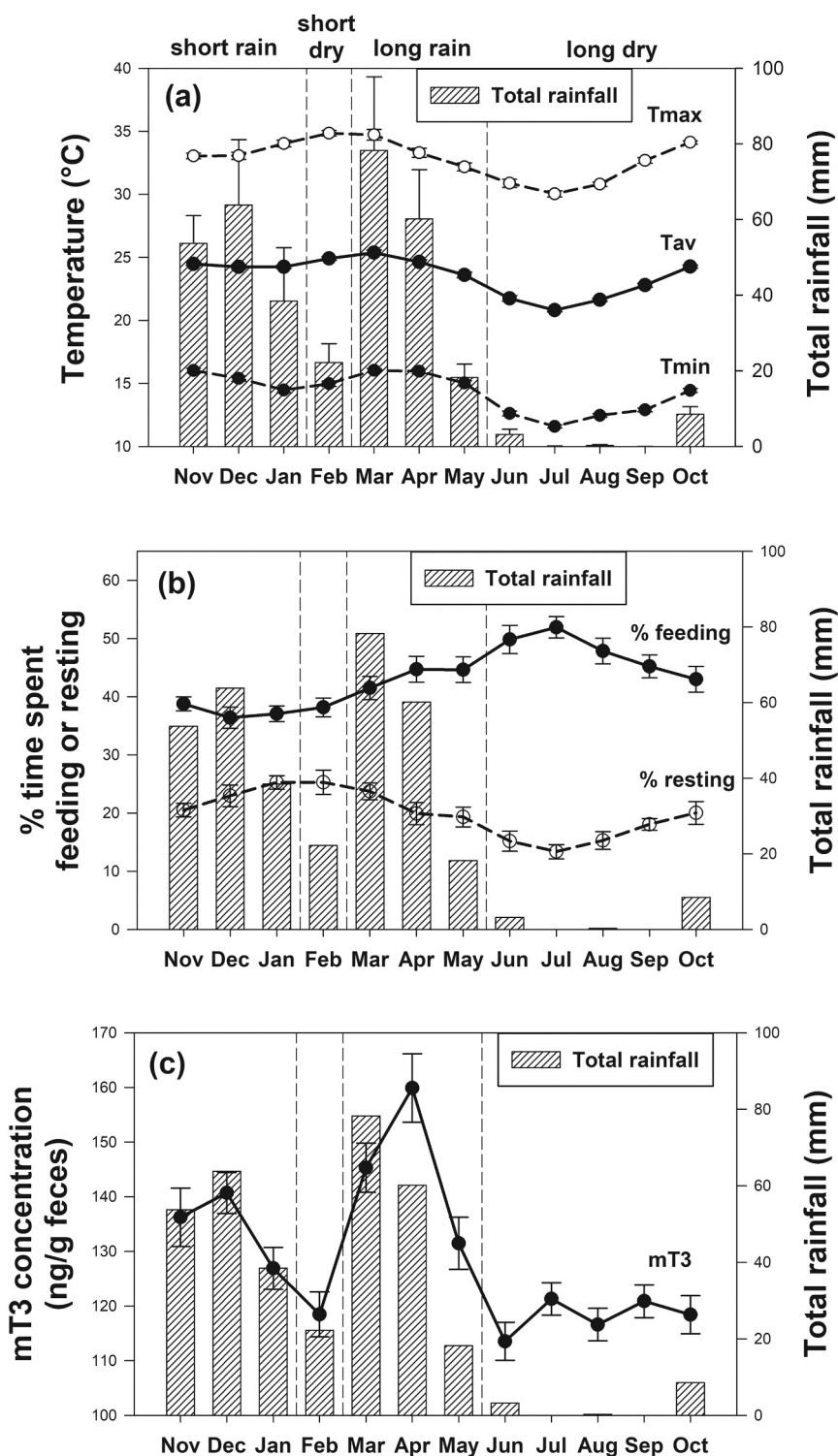


Fig. 1. Monthly and seasonal patterns of (a) total rainfall and temperature (T_{\min} , T_{\max} and T_{av}), (b) percent time spent feeding and resting, and (c) fecal thyroid hormone (mT3) concentrations. Months are ordered according to “hydrological year”, beginning with November, the first month of the wet season (as in, e.g., [Altmann et al., 2002](#)). Each bar graph and point represent the mean and standard error across the 14 years of data used for this study. The four seasons, indicated by text above the graph and separated by gray dashed lines, were defined as follows: short rainy season from October 27th through January 31st, short dry season from February 1st through February 28-29th, long rainy season from March 1st through May 15th, and long dry season from May 16th through October 26th. Note that the total amount of rainfall in May (part of the long rains) is less than the total amount of rainfall in February (the short dry season) because the month of May falls half in the long rains and half in the long dry season. See text for details.

variation in plant phenology that is not fully captured by rainfall and temperature alone. For instance, *Vachellia tortilis* trees produce pods – an important baboon food – during one of the two dry seasons but not the other; similarly, *V. tortilis* and *V. xanthophloea* produce abundant blossoms – another important baboon food – in only one of the two wet seasons. Furthermore, some baboon foods are season-dependent but not rain-dependent, while others are rain-dependent but not season-dependent. For example, *V. tortilis* trees produce pods during the long dry season in almost every year, regardless of rainfall, while grass growth is entirely rain-dependent and is not strictly seasonal. Second, rainfall and temperature are not uniformly correlated as a function of dry versus wet seasons. For instance, the short dry season corresponds to one of the hottest months of the year, while the long dry season includes the coolest months of the year. By including the ‘season’ variability we capture the distinct rainfall-temperature combinations that characterize the ecosystem in a simple and concise way.

We modeled season by first determining the beginning and end of each of the four East African seasons in Amboseli (short rainy, short dry, long rainy, and long dry seasons). Specifically, we determined the break points in rainfall patterns at the beginning and end of these seasons using threshold models (‘Chngptm’ package in R) applied to Amboseli rainfall data from years 2000–2019. These models produced estimates of the timing of the transition between the seasons, with low standard error for the estimate of the end of the long dry season and start of the short rains in October (October 27th (± 2 days)) and the end of the short rains and start of long dry season in May (May 16th (± 7 days)). However, they did not perform as well for determining the start and end of the short dry season: large year to year variability in rainfall patterns produced estimates with high standard errors, with the short dry season estimated to begin on January 21st (± 16 days), and the short dry season estimated to end (and the long rains to begin) on February 27th (± 14 days). We therefore supplemented the threshold analyses with a careful visual inspection of the rainfall patterns between January and March (See Fig. S1). This process led us to define the sole month of February as the short dry season (beginning Feb 1st and ending Feb 28–29th); both dates are well within the standard errors defined by the threshold models.

2.4. Data on female behavior

To link climate to behavior and mT3 concentrations in individual fecal samples, our behavioral analyses focused on individual activity budgets (feeding, resting, walking, and socializing) and the percentage of time spent eating different types of foods. Our behavioral data are derived from 39,292 10-minute focal animal behavioral samples from our 220 adult female subjects (mean \pm SD: 175 \pm 149 samples per female, range 1–797) in all reproductive states. Despite the large number of behavioral samples, they were too sparse at the level of the individual to align well with the opportunistic nature of our fecal sampling. For instance, we might collect a fecal sample for a given female on the first day of a particular month, but not collect a 10-minute behavioral sample on her until the middle of that month, making it impossible to link her individual activities and food choices to her mT3 concentrations. To address this problem, for activity and diet, we used pooled estimates of the percentage of time that females of a given reproductive state, in a given social group for a given season and hydrological year, spent in different activities and ate different food types. In other words, the behavioral data were treated as an aggregate dataset allowing us to estimate the average female's activity and food budget, rather than linking individual behavioral data samples to individual fecal samples. This approach leverages the fact that baboons in a given social group travel through the landscape in a coordinated fashion, encountering similar resources, eating the same foods at the same time, and engaging in similar activities.

2.4.1. Measuring activity budgets

The behavior of all adult females in each group was sampled in random order during focal animal samples. During each 10-minute sample, once per minute, we collected a ‘point sample’ in which we recorded the activity of the focal baboon, categorized as feeding (including food processing), walking while not feeding, resting, or socializing (grooming, being groomed, other social activities; see Altmann, 1974; Alberts et al., 2005). We used these point samples to calculate the total percentage of time spent in each activity for all the females in a given reproductive state (cycling, pregnant, or in post-partum amenorrhea; see Section 2.5. for details) in a given social group, in a given season and hydrological year; thus, we had one data point for how much time pregnant females spent in each of the four activities (feeding, walking, resting, and socializing) in each season of each year, how much time post-partum amenorrhea females spent in each activity in each season of each year, and how much time cycling females spent in these activities in each season and year. Because the four activities added up to 100 % and were necessarily highly correlated with each other, we only used as predictors in our models of mT3 concentrations the two activities likely to have the strongest relationship to metabolic changes: feeding and resting. Because baboons spend more time feeding when food availability and/or food quality are low, ‘time spent feeding’ reflects the cost and effort to acquire energy. ‘Time spent resting’, in contrast, can be used as a means to mitigate the costs of energy acquisition as resting baboons decrease the energy they expend and conserve energy.

2.4.2. Measuring diet composition

When the activity during a given point sample was feeding, the type of food was recorded and whenever possible the species and plant part identified (see Table 6.2 in Alberts et al., 2005 for a list of the main food items eaten by Amboseli baboons). We had one or more records of feeding behavior with an identified food type in 76 % of our 10-minute focal animal samples. We categorized foods into eight major food types, with five types considered high-energy foods: grass blades, fruits, flowers, tree gum, and invertebrates; and three types considered low-energy foods: grass corms, grass blade bases and seed heads, and *Vachellia* seeds (Altmann, 1998). These eight food types constituted 97 % of the total food eaten. We then measured the percentage of time spent eating each food type, pooling across all females in a given reproductive state, in a given social group, in a given season and hydrological year. Because these eight food types constituted nearly 100 % of the diet, the percentage of time spent on the various food types showed high covariance. Consequently, we only considered two food categories in our statistical models: (i) the percentage of feeding time devoted to grass corms (the food with the lowest energy-return relative to the energy invested into their harvest; Altmann, 1998) and (ii) the percentage of feeding time devoted to high-energy foods (i.e., grass blades, fruits, flowers, gum and invertebrates combined).

2.5. Female reproductive state

Data on female reproductive state are recorded on a near-daily basis for all females aged four years and older, regardless of whether they have yet achieved menarche (adulthood). On each observation day, we record the presence of external menstrual bleeding, the color of the paracallosal skin (an indication of pregnancy or recent parturition; Altmann, 1973), and both the size and condition (turgescence, deturgescence) of the sexual swelling (Gesquiere et al., 2007; Gesquiere et al., 2018b). Females were categorized as immature if no sexual swelling had yet been observed for them. We categorized adult females (those that have achieved menarche) as either cycling (exhibiting a cyclical pattern of turgescence of the sex skin, followed by deturgescence and then vaginal bleeding), pregnant (cessation of sexual swellings without evidence of menstruation and followed by subsequent confirmation; see Beehner et al., 2006b), or in the period of lactational or post-partum

amenorrhea (hereafter PPA, lasting from parturition until resumption of cycling). From these data, the timing of four reproductive events was determined: (1) onset of menarche (first day a sexual swelling was observed for the first time), (2) cycling resumption (first day of sexual swelling following post-partum amenorrhea), (3) conception (last day of sex skin turgescence before cessation of cycling), and (4) live birth (day an offspring was born alive, removing stillbirths) (Altmann et al., 1977; Altmann and Alberts, 2003). For our third objective, understanding whether the timing of reproductive events is partially explained by variation in metabolic activity, we focus on the first three of these events (menarche, cycling resumption, and conception) rather than live birth, because the timing of live birth is largely under fetal rather than maternal control (reviewed in Mijovic and Olson, 1996).

2.6. mT3 extraction and radioimmunoassay

mT3 concentrations were measured from fecal samples. Our previous research has shown that mT3 concentrations can be accurately measured in baboon feces, are stable under storage conditions used for steroid hormone analysis, and reflect known biological variation (Gesquiere et al., 2018a).

Fecal sample collection, storage, and sifting were carried out as described previously (Khan et al., 2002; Lynch et al., 2003; see also Gesquiere et al., 2022 for the details on the hormone lab protocols). In brief, immediately after collection of freshly deposited fecal samples from known individuals, these samples were mixed and placed in 95 % ethanol, and kept refrigerated until shipped to the University of Nairobi (every two weeks). In Nairobi, samples were freeze-dried, then sifted to remove the vegetative matter, and stored at -20°C until transported to the US.

In the US, we extracted 0.05 g of the resulting fecal powder sample into 5 ml of 70 % ethanol using a multipulse vortexer for 30 min. After centrifugation of the samples for 25 min at 2300 rpm, the supernatant was collected. The procedure was repeated once, after which the second 5 ml supernatant was combined with the first one and thoroughly mixed. We pipetted 2.5 ml of the ethanol extract, evaporated it and added 250 μl of buffer (T3 standard '0' from the RIA kit), thereby concentrating the samples 10-fold, following earlier protocols (Wasser et al., 2010).

Total mT3 concentrations were determined using the T3 ^{125}I kit (catalog # 06B254216, MP Biomedicals, Costa Mesa, CA), following the supplier's instructions. Per the manufacturer, the primary antibody cross-reacts 100 % with L-triiodothyronine (T3), 0.18 % with L-thyroxine (T4), 0.44 % with 3,5-diiodothyronine, 0.01 % with 3,3',5'-L-triiodothyronine (r-T3), and < 0.01 % for 3,5-diiodotyrosine, phenylbutazone, sodium salicylate, diphenylhydantoin and dicumerol. All samples were run in duplicate, the concentration of each duplicate was averaged, and the results were expressed as ng/g dry fecal matter. The T3 radioimmunoassay was previously validated for use with Amboseli baboon feces (Gesquiere et al., 2018a). The intra-assay coefficients of variation (CV) were 3.22 % ($N = 13$) for a 408 ng/dl fecal pool from captive baboons and 7.9 % ($N = 8$) for a 28 ng/dl fecal pool from wild baboons (any duplicate above 15 % was re-assayed). The inter-assay CVs were 8.66 % ($N = 286$), 6.15 % ($N = 286$) and 4.62 % ($N = 286$) respectively for 50, 100 and 200 ng/dl T3 controls and 13.19 % for the 86 ng/dl fecal pool from wild baboons ($N = 250$).

The period of time between the collection of the fecal sample and the radioimmunoassay varied considerably, from 0.85 to 14.33 years (mean = 7.58 ± 3.52 years). We included a 'storage' variable in our all our statistical models to account for this variation in storage time of our fecal samples.

2.7. Data analysis

2.7.1. Objective 1: visualizing seasonal and annual variation in climate (rainfall and temperature), baboon activity budgets, and mT3 concentrations

We began by visualizing the variation in rainfall, temperature, activity patterns, food eaten, and mT3 concentrations across calendar months, seasons, and years in order to provide a broad context and make decisions about subsequent statistical analyses (Fig. 1, Fig. S1, Fig. S2). To do so, we first calculated, for each hydrological year, the monthly total rainfall, the monthly mean of temperature (T_{\min} , T_{\max} and T_{av}), the monthly percentage of time spent in each activity pooled across all the adult females in our population, and the monthly mean mT3 concentrations pooled across all adult females. We then calculated the mean and standard error for all these variables across our 14 years of data. To visualize seasonal variation in food types eaten, we calculated the percentage of time spent eating each of the eight main food types for all the adult females in our population, pooling all years of data and separating into the four seasons described in Section 2.3.2. (Fig. 2a). Finally, to visualize variation within season between years (Fig. 2b and Fig. S3), we calculated for each season in each hydrological year the mean daily rainfall, mean T_{av} , mean mT3 concentrations, and percentage of time spent eating each food type.

2.7.2. Objective 2: testing environmental and behavioral variables as predictors of mT3 concentrations, accounting for female reproductive state and group size

2.7.2.1. Variation across the hydrological year. We used an information-theoretic approach to analyze sources of variance in mT3 concentrations across the hydrological year. This method is an alternative to null hypothesis testing and is especially appropriate when trying to determine the relative importance of a set of predictors that might influence a response variable (Burnham et al., 2002, 2011; Symonds and Moussalli, 2011).

We first constructed a linear mixed-effect model ("lmer" function of the 'lme4' package of the statistical software R version 4.0.4; Bates et al., 2015) with log of mT3 concentrations in each fecal sample as the response variable. We log transformed the mT3 concentrations to obtain a normal distribution of the model residuals. Ten predictor variables were included in the model: (i) season in which the fecal sample was collected, (ii) mean daily rainfall over the 30 days prior to the collection of the fecal sample, (iii) mean T_{av} over the 30 days prior to the collection of the sample (iv) percentage of time spent feeding, by all the females in the social group that were in the same reproductive state as the female from whom the sample was collected, during the same season and hydrological year of fecal sample collection (hereafter % time feeding), (v) percentage of time spent resting (hereafter % time resting), (vi) percentage of feeding time devoted to corms (hereafter % corms eaten), (vii) percentage of feeding time devoted to high-energy foods eaten (hereafter % high-energy foods), (viii) the reproductive state of the female from whom the fecal sample was collected, (ix) group size on the date of sample collection, and (x) time fecal sample was stored (hereafter storage; see Table 1 for complete descriptions). Our model also included female identity, group identity, and hydrological year as random effects to control for differences between females, groups, and years. Because we found that baboons exhibited low mT3 concentrations in months when ambient temperatures were either extremely hot (Feb and Oct) or cold (Jun-Aug; see Fig. 1), we entered T_{av} as quadratic function, despite our initial prediction that mT3 concentrations will be high when T_{av} is low, and low when T_{av} is high.

Because our population of primarily yellow baboons shows admixture with anubis baboons, we also ran the same model including genetic ancestry as an additional predictor variable on a subset of our fecal samples (4785 of the 7107 fecal samples); these samples were collected

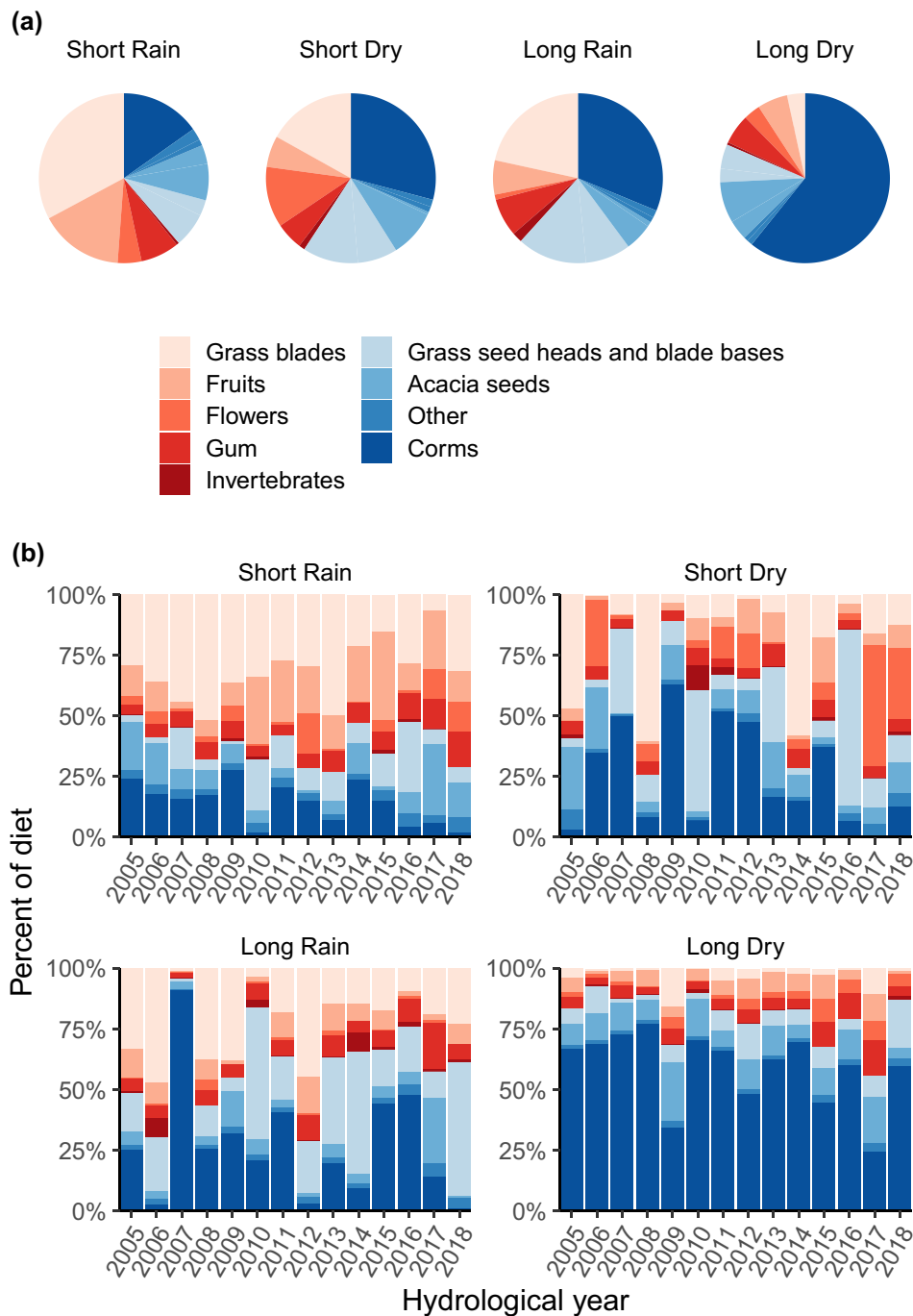


Fig. 2. Variation in the percentage of feeding time of female baboons that is dedicated to each major food type (a) in each of the four seasons, pooled across years and (b) within seasons, showing variation between years. The high-energy food types are represented in red and the low-energy food types are in blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

from the subset of 132 (out of the total 220) females for whom genetic ancestry estimates are available. Genetic ancestry estimates provide a measure of the proportion of each subject's genome estimated to be from *P. anubis* ancestry based on whole-genome resequencing data, such that an estimate of 1 corresponds to unadmixed *P. anubis* ancestry and an estimate of 0 corresponds to unadmixed *P. cynocephalus* ancestry (Vilgalys et al., 2022). We found no evidence that ancestry predicted female mT3 concentrations (see supplementary materials Table S1 and S2), so we did not include ancestry estimates in the models presented in this paper.

To assess collinearity among predictors, we calculated generalized variance inflation factors (GVIFs) with the 'car' package in R. The GVIFs

for the predictor variables included in these models were <5, except for corms which had a GVIF of 5.3 (Fox and Weisberg, 2019).

We evaluated candidate models based on the adjusted Akaike Information Criterion (AIC_c) values, which includes a correction for small sample size and is recommended over AIC (Burnham et al., 2011). We selected the best model as that with the lowest AIC_c value, and when two or more models had an AIC_c difference of <2, we considered the models as equally supported (Burnham et al., 2011). These analyses were conducted using the "dredge" function in the 'MuMIn' package in R.

After the initial information theoretic analysis, we estimated the effect size of each parameter using model averaging as well as their relative importance by calculating the sum of Akaike weights. Both the

averaged estimates and the relative importance for parameters were calculated across all candidate models as our results showed high model selection uncertainty (i.e., the top models had low Akaike weight, see Lukacs et al., 2010). Finally, the variance explained by the fixed and random effects was estimated using the function “get_variance” in the ‘Insight’ package in R.

2.7.2.2. Variation within seasons between years. The four seasons in Amboseli, which are distinct from each other in their temperatures, rainfall patterns, and food types available to baboons, are also highly variable between years: rains sometimes fail to materialize during the nominal short or long rainy seasons, and the short dry season sometimes experiences considerable rain (Fig. S1, Fig. S3). This interannual variation in climatic conditions leads to the possibility that interannual variation in mT3 concentrations within seasons might have different predictors than variation between seasons. To examine this possibility, we built season-specific linear mixed effects models (one for each season), each with the same predictors as in the model examining variation across the hydrological, excluding season: mean daily rainfall, mean T_{av} (as a linear function within each season), % time feeding, % time resting, % corms eaten, % high-energy foods eaten, reproductive state, group size, and storage (Table 1). We again included female identity, group identity and hydrological year as random effects in all models. Candidate models were evaluated based on their AIC_c. The relative importance and model-averaged estimates of the effect sizes for each parameter were calculated as previously described.

2.7.3. Objective 3: testing seasonal patterns of reproduction and variations in mT3 concentrations in the months prior to menarche, cycling resumption, and conception

We first determined the frequency distribution by month of all four reproductive events measured during our study period (2005–2018): (1) onset of menarche (i.e., the onset of a female's first sexual cycle; Fig. S4a), (2) cycling resumption following post-partum amenorrhea (Fig. S4b), (3) conception (Fig. S4c), and (4) live birth (Fig. S4d). To determine if these four reproductive events showed seasonality, we used circular statistics (‘circular’ package in R) and tested for significant differences using the Rayleigh test for nonuniformity of circular data, as performed previously in this population (Janson and Verdolin, 2005; Campos et al., 2017). We determined the r-vector—a parameter used in circular statistics to tests for uniformity of births across the year—in the Amboseli baboons. The r-vector varies from 0 for strictly non-seasonal breeders that give birth throughout the year to 1 for strictly seasonal breeders for whom all births occur within a month (Janson and Verdolin, 2005; Campos et al., 2017; Heldstab et al., 2021). We also graphed the monthly mean concentration of mT3 for all adult female baboons in a circular format for comparison with the four frequency distributions.

We then tested whether female baboon's metabolic activity acted as a cue for three of these four events: menarche (achieving ovarian cycling for the first time), cycling resumption after post-partum amenorrhea, and conception; we did not examine live birth in this context because the timing of live birth is largely under fetal control (Mijovic and Olson, 1996). To determine if menarche, cycling resumption, or conception were preceded by an increase in female mT3 concentrations, we used a piecewise regression analysis. We chose this approach because we predicted that mT3 concentrations would show relatively abrupt changes as females transitioned from one reproductive state to the next. Specifically, we expected mT3 concentrations to increase relatively abruptly as the period of post-partum amenorrhea drew to a close, and females activate their metabolism as their energy balance improved before resuming cycling. We expected similar patterns in the period leading up to menarche and conception.

For this analysis, we determined breakpoints in mT3 concentrations during the 6-month periods that preceded each of these three reproductive events using threshold models (‘Chngptm’ package in R), and we

evaluated whether mT3 concentrations showed systematic changes (increase or decrease) before the breakpoints and/or after the breakpoints. That is, we separated each dataset in two (before/after breakpoint) and ran linear mixed-effect models on each subset of each dataset, with mT3 concentrations as our response variable and days relative to cycle resumption or days relative to conception as our predictor variable. Female ID was also entered as random variable.

3. Results

3.1. Objective 1: variability in climate, activity budget, diet, and hormone concentrations within and between years

As is typical for East African savanna habitats, we found that rainfall was variable across the hydrological year and between years with considerable year-to-year variation during the short rainy, short dry, and long rainy seasons (Fig. 1a, Fig. S3). Temperature also varied within and between years, but to a lesser extent than rainfall (Fig. 1a, Fig. S3). The hottest temperatures occurred in February and March, with maximum daily temperatures in the shade often close to and sometimes exceeding baboons' normal core body temperature of 38 °C. Cooler temperatures were observed during the long dry season in the months of June through August (Fig. 1a) with minimum daily temperatures reaching lower than 10 °C. As is commonly seen in arid tropical environments, temperature varied more within a 24-h. period than between months and years (Altmann et al., 2002).

Female baboons adapted their activity budgets in response to seasonal variation in rainfall and food availability, spending more time feeding and less time resting during the long dry season (Fig. 1b). The increase in time spent feeding during the long dry season reflected the increase in the percentage of feeding time devoted to grass corms (Fig. S2), as corms required a longer processing time than most other food consumed. The percentage of feeding time devoted to high-energy foods (i.e., fresh grass blades, fruits, flowers, gum, and invertebrates) also varied considerably across the hydrological year: it peaked in the short rainy season, was intermediate in the short dry and long rainy seasons, and was lowest in the long dry season, which was characterized by a heavy reliance on corms (>60 % of the diet; Fig. 2a). Baboon diets were also highly variable from year to year, especially in the short dry and the long rainy seasons (Fig. 2b).

Female baboons had higher mT3 concentrations during the two rainy seasons than during the two dry seasons (Fig. 1c). mT3 concentrations also showed strong variation between years, particularly in the two rainy seasons (Fig. S3a, S3c) and the short dry season (Fig. S3b), while mT3 concentrations varied relatively little between years during the long dry season, which represents the most predictable season of the Amboseli year (Fig. S3d).

3.2. Objective 2: females exhibited low mT3 concentrations when they were pregnant, lactating, and during harsh environmental conditions

3.2.1. Variation in mT3 concentrations across the hydrological year

Ranking of the 1024 candidate models predicting variation in mT3 concentrations revealed five with considerable support ($\Delta AIC < 2$) and 11 models with some support ($\Delta AIC < 10$) (Table 2). The five top models all included season, daily rainfall, T_{av}^2 , % corms eaten, % time spent resting, female reproductive state, and storage time. Group size and % high-energy foods eaten were in three and two of the five top models, respectively.

The model averaged parameter estimates and relative importance strongly support that season, daily rainfall, T_{av} , % corms eaten, % time spent resting, and female reproductive state were all important predictors of mT3 concentrations. The relative importance for these 6 parameters were all equal to 1, and their 95 % CIs did not overlap zero (Table 3). In contrast, time in storage and group size received less support for being important predictors of mT3 concentrations, as despite

Table 2

Factors predicting fecal thyroid hormone concentrations across the hydrological year.

Fixed effects	AIC _C	ΔAIC _C	Akaike weight
ReproState^a + Season + Rain^b + T_{av}² + Corms^c + Resting^d + GpSize^e + Storage	−7331.21	0.00	0.23
ReproState + Season + Rain + T_{av}² + Corms + Resting + Storage	−7330.39	0.82	0.15
ReproState + Season + Rain + T_{av}² + Corms + Resting + GpSize + Storage + HEF^f	−7330.12	1.09	0.13
ReproState + Season + Rain + T_{av}² + Corms + Resting + Storage + HEF	−7329.29	1.91	0.09
ReproState + Season + Rain + T_{av}² + Corms + Resting + GpSize + Storage	−7329.22	1.99	0.09
ReproState + Season + Rain + T _{av} ² + Corms + Resting + GpSize + Storage + Feeding ^g	−7328.46	2.75	0.06
ReproState + Season + Rain + T _{av} ² + Corms + Resting + Storage + Feeding	−7328.21	3.00	0.05
ReproState + Season + Rain + T _{av} ² + Corms + Resting + GpSize	−7328.14	3.06	0.05
ReproState + Season + Rain + T _{av} ² + Corms + Resting + GpSize + Storage + Feeding + HEF	−7327.37	3.83	0.03
ReproState + Season + Rain + T _{av} ² + Corms + Resting + Storage + Feeding + HEF	−7327.30	3.91	0.03
ReproState + Season + Rain + T _{av} ² + Corms + Resting + GpSize + HEF	−7326.22	4.98	0.02
ReproState + Season + Rain + T _{av} ² + Corms + Resting + GpSize + Feeding	−7326.19	5.02	0.02
ReproState + Season + Rain + T _{av} ² + Corms + Resting	−7325.32	5.88	0.01
ReproState + Season + Rain + T _{av} ² + Corms + Resting + GpSize + Feeding + HEF	−7325.28	5.92	0.01
ReproState + Season + Rain + T _{av} ² + Corms + Resting + HEF	−7324.26	6.94	0.01
ReproState + Season + Rain + T _{av} ² + Corms + Resting + Feeding	−7323.38	7.83	0.00

Fixed effects, Akaike information criteria (AIC_C), delta AIC_C and Akaike weights (Burnham et al., 2002) are provided for models with ΔAIC_C < 10. ΔAIC_C was calculated as the difference between a given model and the best model. Akaike weights reflect the probability that a given model was the best model in the candidate model set. Models are sorted in order of their Akaike weights and those with a ΔAIC_C < 2 are in bold.

^a ReproState = female reproductive state.

^b Rain = mean daily rainfall.

^c Corms = % corms eaten.

^d Resting = % time spent resting.

^e GpSize = group size.

^f HEF = % high-energy foods eaten.

^g Feeding = % time spent feeding.

having relative importance > 0.6, they both had 95 % CIs that overlapped zero (Table 3). % high-energy foods eaten and % time spent feeding did not predict mT3 concentrations across the hydrological year, as suggested by their low relative importance < 0.4 and 95 % CIs overlapping zero.

Concentrations of mT3 were lower in pregnant and PPA females, when daily rainfall was low, when ambient temperature was either at the low end or high end of the distribution of observed temperatures, and when females spent less time resting and ate more corms (Table 3). Furthermore, after correcting for these variables, mT3 concentrations were higher during the long dry season than during the other seasons, and significantly so compared to the short rainy and short dry seasons. In other words, although absolute mT3 concentrations were lower during the long dry season than during any other season, they were higher than predicted given how much time the baboons spend eating corms in this season and taking into consideration the relatively low availability of rain-dependent foods (which are generally associated with higher mT3 concentrations). Overall, these fixed effects explained 11.4 % of the

variance in mT3 concentrations, while the random effects explained 15.7 % (Fig. S5).

3.2.2. Variation in mT3 concentrations within seasons between years

Overall, our results within seasons between years were largely consistent with the results across the hydrological year, showing the importance of female reproductive state, daily rainfall, and % time spent resting in predicting mT3 concentrations (see Tables S3 and S4 in supplementary materials). Interestingly, we also found that the % of high-energy foods eaten predicted mT3 concentrations during the short dry season, with higher mT3 concentrations occurring when females ate a higher percentage of high-energy food (Tables S3 and S4). The variance explained by the fixed effects between years within a given season was in general lower than the variance explained across the hydrological year, with the exception of the long rainy season (Fig. S5 in supplementary materials). Within seasons, random effects, especially hydrological year, explained a larger part of the variance in mT3 concentrations than the fixed effects, indicating considerable year-to-year variation in mT3 concentrations that was not captured by our fixed effects (Fig. S5).

3.3. Objective 3: females showed weak reproductive seasonality and their mT3 concentrations increased prior to menarche and cycling resumption but not conception

Even though female baboons in Amboseli were observed to cycle, conceive, and give birth in every month, our analysis confirmed that they showed weak reproductive seasonality (Fig. 3). Menarche occurred more often than expected during the two rainy seasons, from October through January, and in May (vector length $r = 0.1854$, $p < 0.001$, $N = 153$; see Fig. 3a). Similarly, resumption of cycling occurred at a higher frequency than expected from October through December and in April and May ($r = 0.1055$, $p < 0.001$, $N = 668$; see Fig. 3b). Conception occurred more often than expected from January through June ($r = 0.1469$, $p < 0.001$, $N = 773$; see Fig. 3c). And not surprisingly, because live birth usually occurred 6 months after conception, we found a peak in live birth from July through December ($r = 0.1621$, $p < 0.001$, $N = 628$; see Fig. 3d). A similar visualization, in a circular format, of the monthly mean concentration of mT3 showed a relatively close correspondence to the monthly frequency distributions of menarche and cycling resumption but not to the monthly distributions of conception or live birth (Fig. 3e). Specifically, we saw that two of the peak frequencies observed for menarche and cycling resumption (in November–December and in April) corresponded to two peaks in mT3 concentrations (Fig. 3).

Our linear models revealed that mT3 concentrations showed a statistically significant increase in the data subset beginning 21 days prior to menarche ($\beta = 2.764$, $p = 0.045$, Fig. 4a), but not in the data subset prior to that breakpoint. In other words, approximately 3 weeks before they achieve menarche, immature female baboons exhibit an abrupt increase in mT3 concentrations. Similarly, we found a statistically significant increase in mT3 concentrations beginning 27 days prior to cycling resumption, although the magnitude of this increase was smaller than for menarche ($\beta = 0.919$, $p = 0.011$, Fig. 4b). However, no increase in mT3 concentrations was observed in the weeks prior to conception ($\beta = 0.133$, $p = 0.382$, Fig. 4c).

4. Discussion

Our analyses reveal considerable variation in mT3 concentrations for female baboons, both within and between years. This variation parallels variation in rainfall and temperature observed in Amboseli, as well as substantial changes in the activity budget and diet of female baboons within and between years. Female mT3 concentrations also varied considerably as a function of reproductive state: mT3 concentrations were lower in pregnant and PPA females than in cycling females,

Table 3

Relative importance and model-averaged estimates for the parameters predicting mT3 concentrations across the hydrological year.

Fixed effects ^a	Relative importance	Estimate ^c	Lower 95 % ^c	Upper 95 % ^c	Interpretation
(intercept)		119.950	99.759	144.227	
ReproState:	1.00				
P vs. C ^b		−3.043	−5.154	−0.885	mT3 3.0 % lower in pregnant than in cycling females
PPA vs. C ^b		−9.065	−10.827	−7.269	mT3 9.1 % lower in lactating than in cycling females
Season:	1.00				
LR vs. LD ^c		1.926	−2.133	6.153	—
SD vs. LD ^c		−12.084	−15.671	−8.344	mT3 12.1 % lower in SD than in LD season
SR vs. LD ^c		−8.791	−11.981	−5.485	mT3 8.8 % lower in SR than in LD season
Daily rain	1.00	4.196	3.422	4.975	mT3 4.2 % higher for each 1 mm increase in mean daily rain
Temperature	1.00				
T _{av}		144.343	−20.467	650.675	—
T _{av} ²		−76.628	−89.467	−48.140	mT3 lower when T _{av} is low or high
% corms eaten	1.00	−0.253	−0.317	−0.188	mT3 2.5 % lower for each 10 % increase in corms eaten
% time resting	1.00	0.340	0.180	0.499	mT3 3.4 % higher for each 10 % increase in time spent resting
Storage	0.84	1.536	−0.352	3.460	—
Group size	0.62	−0.064	−0.190	0.062	—
% HEF eaten ^d	0.37	−0.014	−0.072	0.044	—
% time feeding	0.27	0.005	−0.081	0.091	—

Fixed effects are sorted in order of their relative importance, using their sum of weights (SW). Variables above the dashed line have 95 % CI that do not overlap 0 and are significant predictors of mT3 concentrations, while the parameters below the line have 95 % CI that overlap 0 and do not significantly predict mT3 concentrations. Note that a variable may be listed above the line despite some of its categories having estimates with CI overlapping 0 (e.g. LR vs. LD).

^a For all categorical variables, the second category listed was the base level.

^b C = cycling; P = pregnant; PPA = post-partum amenorrhea.

^c LR = long rainy season; LD = long dry season; SD = short dry season; SR = short rainy season.

^d HEF = high-energy foods.

^e The Estimate and CI columns have been antilogged, subtracted by 1, and then multiplied by 100 to indicate the percent change in mT3 concentrations associated with an increase of one unit of the predictor variable. The intercepts were antilogged only.

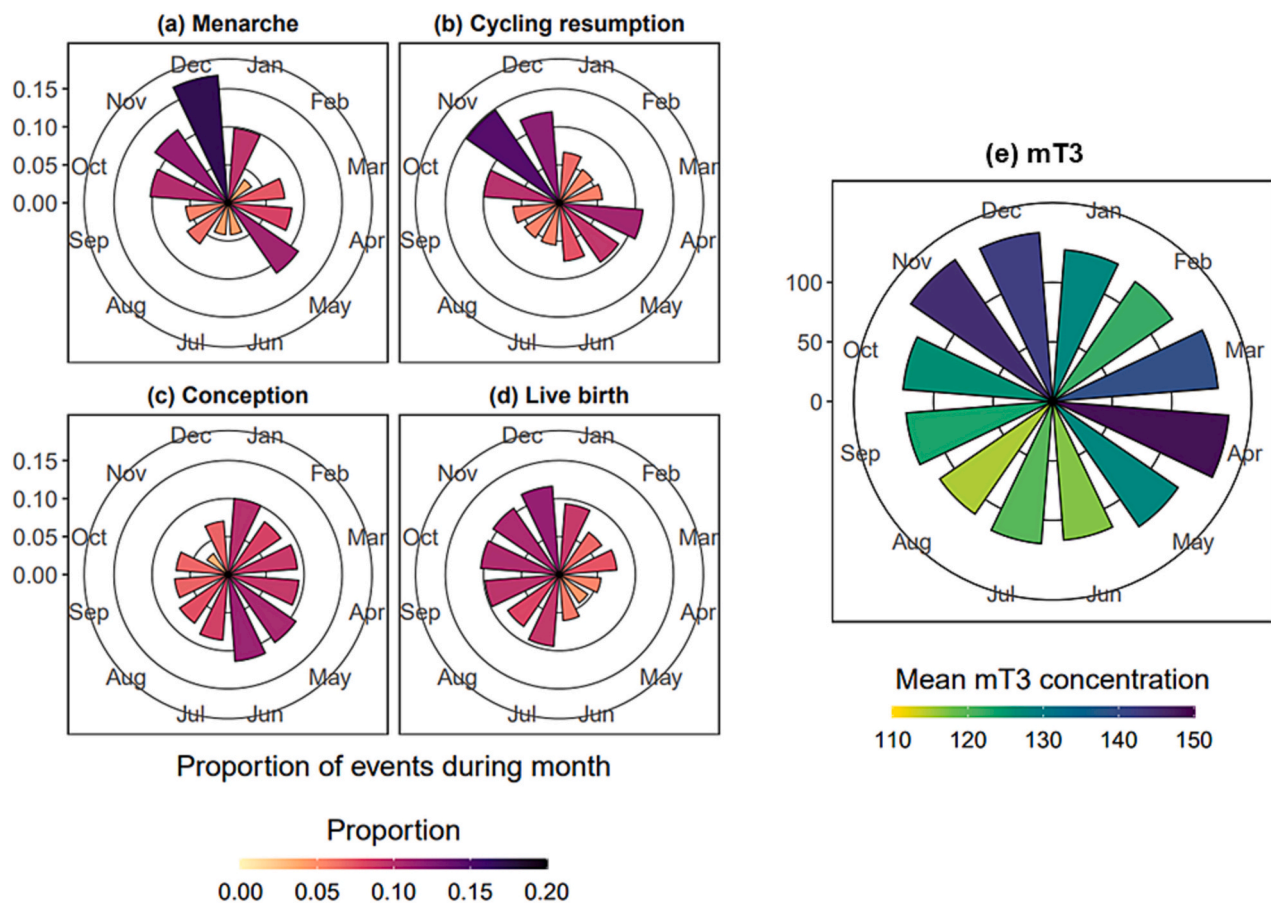


Fig. 3. Circular histograms showing the proportion of (a) menarche (b) cycling resumption (c) conception, and (d) live birth in Amboseli baboons by month from 2005 to 2018. Panel (e) represents the monthly mean concentration of mT3 for all female baboons during the same time period, represented in a circular format for comparison with the four frequency distributions on the left.

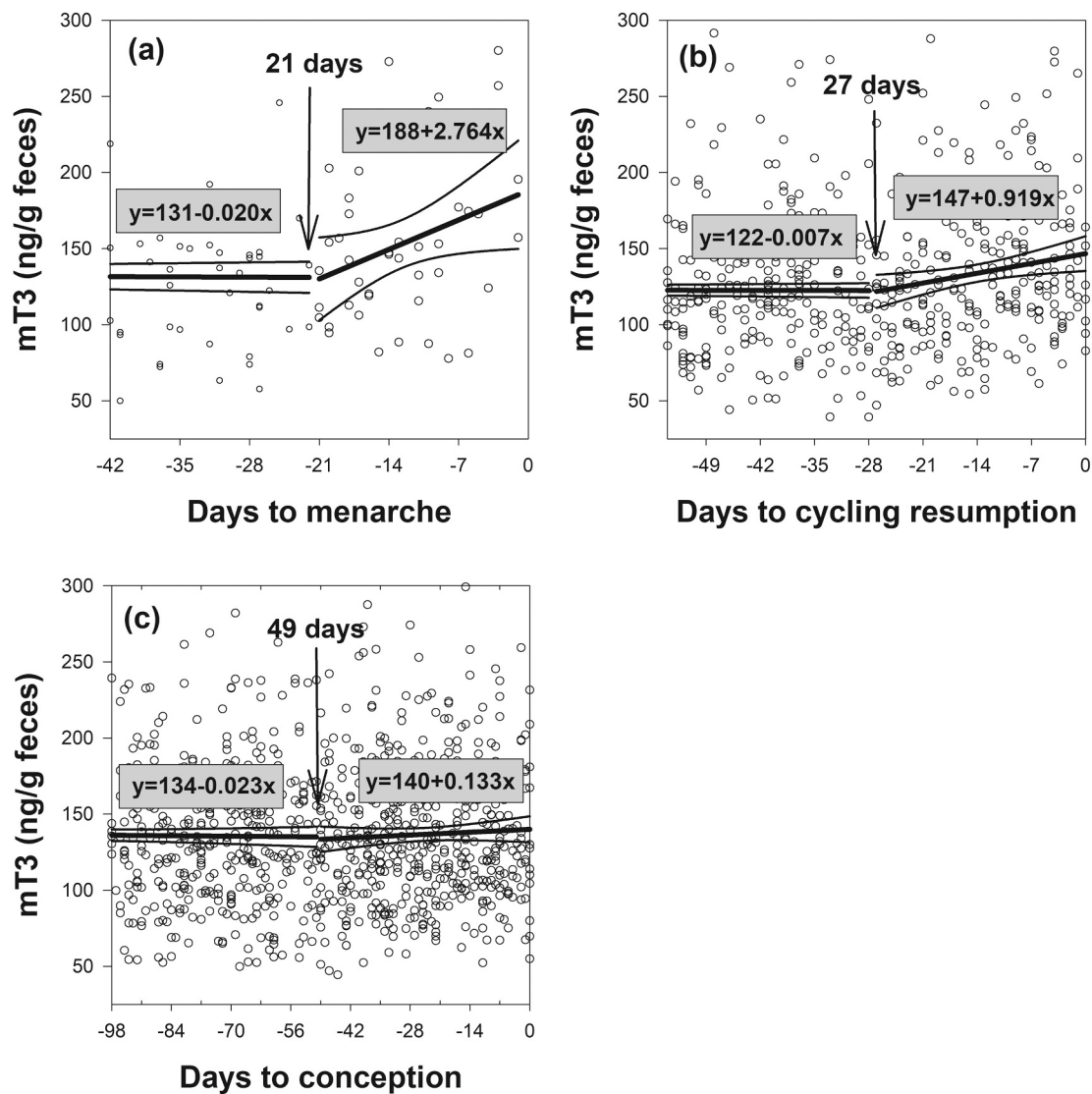


Fig. 4. Variation in mT3 concentrations in the days prior to (a) menarche, (b) cycle resumption and (c) conception. Each point represents a fecal sample. Changes in slope of mT3 concentrations have been identified using threshold models ('Chngptm' package in R, see methods for more details). Breakpoints in mT3 patterns are indicated by arrowhead. We found a change in slope 21 days before menarche, 27 days before cycling resumption and 49 days before conception. Regression lines of the variation in mT3 concentrations before and after the breakpoint and 95 % CIs are included in the graphs. For visualization purposes we do not show the full 6-month period prior to the event but instead zoom in each graph on the breakpoints.

suggesting a reduction in metabolism, possibly to conserve energy (Prentice and Goldberg, 2000; Gesquiere et al., 2018a).

When we tested which environmental and behavioral variables best predicted mT3 concentrations in female baboons, we found that rainfall, temperature, females' time resting, and females' time feeding on corms all predicted mT3 concentrations across the hydrological year. Specifically, mT3 concentrations were lower when rainfall was low and during the hottest and coolest times of the year in Amboseli. mT3 concentrations were also lower when females spent less time resting and devoted more of their feeding time to grass corms. Within seasons, interannual variation in mT3 concentrations during the two rainy seasons was best predicted by rainfall and time spent resting, and during the short dry season, it was best predicted by time spent feeding on high-energy foods.

In spite of the strong environmental seasonality experienced by female baboons in Amboseli, and the clear energetic challenges this seasonality imposes, females reproduced in all months of the year, showing only weak (but statistically significant) reproductive seasonality. Onset of cycling (menarche or resumption) was preceded by an increase in mT3 concentrations, reflecting an activation of female metabolism,

suggestive of the restoration of female energy balance. In contrast, the timing of conceptions showed no relationship with variation in mT3 concentrations.

4.1. Female mT3 concentrations are lowest during the two dry seasons

Our analyses revealed that both environmental factors and changes in females' behavior and diet predicted mT3 concentrations. During periods of low rainfall – particularly the long dry season but also to some extent the short dry season – plant growth is generally reduced and food is scarcer than in the rainy seasons. At these times, baboons switched their diet from high-energy foods (e.g., grass blades, flowers, and fruits) to grass corms (Fig. 2; Alberts et al., 2005). Grass corms are considered a low profitability food, as they require extensive harvesting time relative to the nutrients they provide (Altmann et al., 1987; Byrne et al., 1993; Altmann, 1998), and feeding on corms is associated with an overall increase in the time spent feeding and a decrease in the time spent resting (Fig. 1; Alberts et al., 2005; Altmann, 2009).

Concentrations of mT3 reached an absolute minimum during the

long dry season (Fig. 1c), probably because of an increase in energy expenditure combined with a decrease in energy intake when many rain-dependent foods become unavailable and baboons switch to feeding on grass corms. These low mT3 concentrations during the long dry season also suggest that despite the increase in feeding time during this season, female baboons are not able to fully compensate for the decrease in food quantity and quality, and their energy intake fails to keep pace with their energy expenditure. In response to these challenges, female baboons presumably downregulate their T3 secretion and decrease their metabolic activity as a mechanism to conserve energy. These results are consistent with data reported in several other studies of mammals showing that mT3 concentrations are reduced when food availability is low (Wasser et al., 2010; Ayres et al., 2012; Joly et al., 2015; Cristobal-Azkarate et al., 2016; Schaebs et al., 2016; see also review by Chatzi-tomaris et al., 2017).

At the same time, our statistical analyses indicate that, although absolute mT3 concentrations are lowest during the long dry season, they are relatively higher during this season – controlling for other predictor variables – than during the short dry and short rainy seasons (Table 3). In other words, although absolute mT3 concentrations are lower during the long dry season than during any other season, they are higher than expected given the baboons' activity budgets and food choices. This result suggests the existence of energy-conserving strategies on the baboons' part during the long dry season that we have not yet fully described, and that bear further investigation. These energy-conserving strategies may be important for survival during the severe droughts that sometimes occur in Amboseli (Lea et al., 2015; Okello et al., 2016), and may therefore contribute to the observation that baboons (like most long-lived primates) are relatively “buffered” against environmental variation compared to non-primate mammals (Morris et al., 2011; Campos et al., 2017).

Female baboons also experienced relatively low mT3 concentrations during the short dry season, despite having a diet that was similar to their diet during the long rainy season, except with a higher proportion of high-energy foods. These lower mT3 concentrations may be a consequence of the reduced time spent feeding that we observed during the short dry season compared to the long rainy season. Alternatively, or in addition, the relatively hot temperatures found during the short dry season may force females to reduce activity levels (including feeding time) in order to reduce T3 secretion and hence thermogenesis (Ocobock, 2016; Thompson et al., 2017). This explanation is supported by our results showing that mT3 concentrations were lower when temperatures were hotter. These results are in line with other studies that have demonstrated a reduction in T3 concentrations when temperatures are elevated (Silva, 2003; Kahl et al., 2015; Thompson et al., 2017; Hunnink et al., 2020).

4.2. Female mT3 concentrations are not upregulated by colder temperatures

In contrast to studies in other primates and other endotherms, which often demonstrate an increase in thyroid hormone secretion and basal metabolic rate (BMR) in response to lower temperatures, our data showed that females had lower mT3 concentrations in periods of colder weather (Silva, 2003; Ocobock, 2016; Thompson et al., 2017). This result may appear surprising because thyroid hormones have important roles in thermogenesis and thermoregulation and are expected to increase in cold temperatures to produce heat (Silva, 2003, 2006; Iwen et al., 2018). However, thyroid hormones increase thermogenesis by accelerating ATP turnover and reducing the efficiency of ATP synthesis; heat production is therefore an energetically costly mechanism (Silva, 2003; Iwen et al., 2018). In our study, the coldest temperatures were observed during the long dry season, when high-energy foods were limited and females relied heavily on corms. Therefore, the relatively low mT3 concentrations we observed at the coldest temperatures could be an adaptive mechanism by which female baboons conserve their

energy in this period of food shortage. This interpretation is supported by several studies, including on red deer, Alpine ibex, Shetland ponies, moose, and muskox, which show that animals exposed simultaneously to low temperature and food shortage show evidence of hypo-metabolism, low BMR, or low mT3 concentrations (Arnold et al., 2004; Signer et al., 2011; Brinkmann et al., 2014; Græslie et al., 2020; Desforges et al., 2021).

4.3. Random effects of hydrological year and group identity explain a substantial proportion of the variance in mT3 concentrations

In our models of both variation across the hydrological year and variation between years, hydrological year explained considerable variation in mT3 concentrations (Fig. S5). Hydrological year was a particularly strong predictor of mT3 concentrations during the short dry season and the two rainy seasons. We suspect the magnitude of this effect reflects the considerable yearly variation in plant phenology or in plant nutritional content that is not accounted for by variation in total rainfall alone. For instance, in some years tortilis trees (*V. tortilis*) and fever trees (*V. xanthophloea*) produce abundant blossoms while in other years they produce fewer blossoms, and the timing of their blossoming also varies somewhat from year to year (Altmann, 1998; Alberts et al., 2005). This variability may depend on the temporal patterning of rainfall rather than its total amount (Bronikowski and Altmann, 1996; Bronikowski and Webb, 1996). Additionally, germination, leaf greening, and flowering are likely influenced in complex ways by a combination of variation in rainfall, temperature, and solar radiation (Rathcke and Lacey, 1985; Adole et al., 2016). We lack sufficiently detailed data on variation in phenological timing in Amboseli or its predictors to probe this question further in this dataset, but it raises important questions for future research.

We also found that the identity of the social group in which the female lived explained some variation in mT3 concentrations, particularly during the long dry season. Social groups are characterized by variation in both group size and home ranges. Group size is known to impact intra- and intergroup competition: larger groups experience more within-group competition for food resources than smaller groups, imposing greater energetic costs due to increased foraging time and distances travelled, while smaller groups are energetically constrained by between-group competition (Markham et al., 2015; Markham and Gesquiere, 2017). However, in our analyses group size was included as a fixed effect and did not appear to predict mT3 concentrations. Our baboon study groups also occupied different home ranges at different points in time (Markham et al., 2013), and because home ranges inevitably vary somewhat in the quality and quantity of food available, considerable variation in behavior, diet, and energy metabolism can be expected within a given species or a given population (Altmann, 1998; Chapman et al., 2002; Struhsaker, 2008; reviewed in Chapman and Rothman, 2009). For example, blue monkeys (*Cercopithecus mitis*) occupying areas of lower food richness spent more time feeding, ate lower quality foods, moved less (to conserve energy), and had longer interbirth intervals as a consequence of their lower energy intake compared to groups in the same population but living in areas with higher food richness (Butynski, 1990). Unfortunately, we lack sufficiently detailed data on differences in home range quality among Amboseli baboon study groups to measure its effects directly, but our results suggest that this will be a valuable future direction.

4.4. Onset of cycling but not conception is preceded by an increase in mT3 concentration

Despite births occurring in all months of the year, we confirmed previous findings that female baboons showed measurable although weak seasonality in their reproduction (Alberts et al., 2005; Janson and Verdolin, 2005; Campos et al., 2017).

Menarche and cycling resumption were preceded by an increase in

mT3 concentrations, reflecting an activation of female metabolism in the weeks prior to the onset of cycling. This suggests that female baboons' energy balance was restored prior to cycling onset, and that baboons, like humans and chimpanzees, require a positive energy balance before the onset of sexual cycling (Valeggia and Ellison, 2004, 2009; Emery Thompson et al., 2012). These results are also in line with previous reports that female baboons in Amboseli show an increase in female body fat score and a decrease in fecal glucocorticoid concentrations in the months prior to cycling resumption (Gesquiere et al., 2018b) – a likely signal of restored energy balance (Valeggia and Ellison, 2009; reviewed in Dallman et al., 2004).

By contrast, the timing of conception appears relatively independent of changes in female metabolism and energy balance, as we did not observe changes in mT3 concentrations in the weeks prior to conception. This is consistent with our previous findings in female baboons showing that the duration of ovarian cycling prior to conception was relatively insensitive to environmental variation, and that no changes in fecal glucocorticoid concentrations preceded conception (Gesquiere et al., 2018b).

5. Conclusion

In this study we sought to understand how female baboons in Amboseli modify their behavior and regulate their metabolism in the face of the strong environmental variability they experience. We also sought to understand how these females reproduce in all months of the year, showing only weak reproductive seasonality.

Our results revealed that the seasonally harsh environmental conditions experienced in Amboseli imposed clear energetic challenges to baboon females both across the hydrological year and within seasons, despite their diversified diet and behavioral flexibility. Females showed a reduction in their metabolic activity (lower mT3 concentrations) – suggesting that they were in relatively negative energy balance – when rainfall was low, temperatures were cold or hot, when they ate more corms, and when they rested less – conditions all found during the dry seasons, which comprise a full six months of each year. Our results also suggested that the moderate reproductive seasonality observed in female baboons was mediated by changes in female metabolism, reflecting changes in their energy balance. Indeed, we found that menarche and cycle resumption were preceded by an increase in mT3 concentrations.

These findings offer new insights on the effects of environmental and behavioral variables in regulating females' metabolism and their energy balance and its role in the initiation of reproduction. This study also underlined the importance of fecal mT3 concentrations as a valuable tool to answer questions related to metabolism and energy balance in wild populations.

CRedit authorship contribution statement

Laurence R. Gesquiere: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Christine Adjangba:** Investigation, Data curation. **Tim L. Wango:** Resources, Data curation. **Vivian K. Oudu:** Resources, Data curation. **Raphael S. Mututua:** Resources, Data curation. **J. Kinyua Warutere:** Resources, Data curation. **I. Long'ida Siodi:** Resources, Data curation. **Fernando A. Campos:** Writing – original draft, Visualization, Formal analysis. **Elizabeth A. Archie:** Writing – review & editing, Writing – original draft, Funding acquisition. **A. Catherine Markham:** Writing – review & editing, Funding acquisition, Conceptualization. **Susan C. Alberts:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.yhbeh.2024.105505>.

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