Minimally invasive biomarkers in human and non-human primate evolutionary biology: Tools for understanding variation and adaptation

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Funding information
CIFAR Azrieli Global Scholars Program

Abstract

Background: The use of minimally invasive biomarkers (MIBs – physiological biomarkers obtained from minimally invasive sample types) has expanded rapidly in science and medicine over the past several decades. The MIB approach is a methodological strength in the field of human and non-human primate evolutionary biology (HEB). Among humans and our closest relatives, MIBs provide unique opportunities to document phenotypic variation and to operationalize evolutionary hypotheses.

Aims: This paper overviews the use of MIBs in HEB. Our objectives are to (1) highlight key research topics which successfully implement MIBs, (2) identify promising yet under-investigated areas of MIB application, and (3) discuss current challenges in MIB research, with suggestions for advancing the field.

Discussion and Conclusions: A range of MIBs are used to investigate focal topics in HEB, including energetics and life history variation/evolution, developmental plasticity, and social status and dominance relationships. Nonetheless, we identify gaps in existing MIB research on traits such as physical growth and gut function that are central to the field. Several challenges remain for HEB research using MIBs, including the need for additional biomarkers and methods of assessment, robust validations, and approaches that are standardized across labs and research groups. Importantly, researchers must provide better support for adaptation and fitness effects in hypothesis testing (e.g., by obtaining complementary measures of energy expenditure, demonstrating redundancy of function, and performing lifetime/longitudinal analyses). We point to continued progress in the use of MIBs in HEB to better understand the past, present, and future of humans and our closest primate relatives.
1 | INTRODUCTION

Minimally invasive biomarkers (MIBs) are physiological biomarkers obtained from sample types that minimize degree of disturbance to subjects or participants. Over the past several decades, MIB approaches have advanced considerably and have become widely utilized in the sciences and medicine. In the sciences, MIB application is flourishing in disciplines as diverse as nutrition science (Picó et al., 2019), neurobiology (Clow & Smyth, 2020), and conservation biology (Narayan, 2013). In medicine, MIBs are increasingly measured in surveillance and diagnostic capacities, with a desire to replace and one day eliminate more invasive sampling approaches, such as venipuncture and biopsy tissue collection (Metzler, 2010; Wang et al., 2017).

The field of human and non-human primate evolutionary biology (HEB) has been a longtime leader in MIB research (for early examples of this work, see Brockman et al., 1995; Campbell, 1994; Ellison, 1988; Knott, 1997; Summers et al., 1983; Worthman & Stallings, 1997). In many respects, this position of leadership has emerged as a necessity for carrying out respectful and sustainable research with some of the world’s most vulnerable human and comparative non-human primate (NHP) populations. Researchers in HEB currently measure MIBs among humans and their close primate relatives in diverse contexts and in countries ranging quite literally from “A” (Argentina: e.g., Corley et al., 2021) to “Z” (Zimbabwe: e.g., Campbell & Mbizo, 2006).

The potential advantages of applying MIBs in HEB research are many. Most obviously, MIBs can be obtained with low burden to participants and minimal disruption to their everyday lives, often providing the only possible approach for implementing research designs with direct physiological measures (e.g., among young children, populations with cultural aversions such as blood taboos, or wild NHPs). The use of MIBs can also increase overall participation rates, facilitate repeat-measures sampling, and improve statistical power by minimizing potential confounds in physiological analyses (e.g., by avoiding acute stress responses). Importantly, MIB approaches provide HEB researchers with a suite of alternative sampling methods that can be strategically applied based on study population preference and a range of other context-specific factors, including practical considerations such as relative financial cost, biohazard risk, biomarker stability, and required specialization of sample collection/handling (see Gildner, 2021a for a decision-making flow chart example).

The most common sample types currently used for MIB application in HEB are saliva, urine, feces, hair, milk, and finger-prick dried blood spots (DBS). An overview of these six key sample types is provided in Table 1. Each is unique in its utility. As discussed above, there is no best sample type for all HEB applications, and researchers must select the approach that best suits their specific research questions and context. In-depth methodological descriptions, critiques, and comparisons of sample types are beyond the scope of this paper but are provided elsewhere (e.g., Behringer & Deschner, 2017; Lindau & McDade, 2008).

The range of topics being addressed by HEB researchers using MIBs is wide and expanding. Table 2 highlights some of the physiological systems and traits/evolutionary questions that are being investigated, as well as many of the MIBs that are being utilized. An extended table that includes additional MIBs and individual study entries, species names, and other details is provided in Table S1. At the fundamental level, MIBs are employed to document variation in key aspects of human and comparative NHP biology and phenotype (e.g., reproduction, immune function, stress, and aging). Increasingly, MIBs are also used to operationalize and test hypotheses directly relating to human evolution.

This paper provides an overview of the use of MIBs in HEB. Our aim is not to provide a comprehensive review of this expansive area of research. Rather, we have three more specific objectives: (1) To highlight key research topics which successfully utilize MIBs; (2) To identify promising yet under-investigated areas of MIB application; (3) To discuss current challenges in MIB research, with suggestions for advancing the field. Our focus is on the topics that MIBs are utilized to address, not on the range of potential applications for specific biomarkers. Included evolutionary topics were chosen based on implications for the field, coverage in the literature, and our own research interests. For recognition of the broader range of evolutionary topics being explored using MIBs in HEB, see Table 2.

2 | KEY HEB TOPICS INVESTIGATED USING MIBS

2.1 | Energetics and life history variation/evolution

Much has been written on human and comparative NHP energetics and their importance for understanding human phenotypic variation and evolutionary processes (e.g., Ellison, 2017; Emery Thompson, 2017; Pontzer, 2017; Urlacher et al., 2019a). This area of research is deeply rooted in life history theory. Life history theory is built on the premise that organisms evolve under selective pressure to allocate a limited supply of
| Common with NHPs | Time frame of MIB production reflected | Major advantages | Major disadvantages | Key references |
|-----------------|---------------------------------------|-----------------|--------------------|---------------|
| Urine           | Yes                                   | h-4 days        | • Readily available<br>• Can be collected from all ages<br>• Supports repeated sampling in quantity<br>• Low-risk and inexpensive collection process | • Must be frozen during transport and storage<br>• Unsuitable for detecting some short-term hormone patterns (e.g., diurnal rhythms)<br>• Values must adjust for concentration of urine | (Behringer et al., 2017a; Gildner, 2021b; Higham et al., 2020) |
| Saliva          | No                                    | <1–5 min        | • Supports repeated sampling over short intervals<br>• Low-risk and inexpensive collection process | • Must be frozen during transport and storage<br>• Viscous samples can be difficult to pipette<br>• High internal contamination potential with blood and food debris | (Behringer et al., 2017b; Rosenbaum et al., 2018) |
| Milk            | No                                    | 30 min-2 h      | • Low-risk and inexpensive collection process<br>• Unique for providing direct information on mother-infant relationships | • Variation due to collection time and sample volume not easy to control for | (Muletz-Wolz et al., 2019; Quinn, 2021) |
| Feces           | Yes                                   | 12–72 h         | • Time-integrated levels ranging from several hours to several days<br>• Relatively easy to collect from NHPs | • Must be frozen during transport and storage<br>• Moderate disease transmission risk<br>• High purification equipment costs for some analyses | (Bahr et al., 2000; Murray et al., 2013; Ziegler & Wittwer, 2005) |
| DBS             | No                                    | min–hours       | • Finger prick for small volume of blood<br>• No centrifugation or immediate freezing required<br>• Low external contamination risk from filter paper cards | • Additional training requirements<br>• Validation required to demonstrate values reflect serum/plasma | (McDade et al., 2007a; Valeggia, 2007) |
| Hair            | Yes                                   | Up to 3 months  | • Long-term biomarker index<br>• Storage at room temperature long-term<br>• Low-risk and inexpensive collection process | • Not always available from all individuals<br>• Difficult to distinguish between external and internal exposure<br>• Limited reference values | (Esteban & Castaño, 2009; Gao et al., 2013) |
| MIB                              | Sample type | Species | Physiological system | Investigated trait/evolutionary topic                                                                 | Example studies                                                                 |
|----------------------------------|-------------|---------|----------------------|-------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| 8-hydroxy-2′-deoxyguanosine (8-OHdG) | Urine       | Humans  | DNA repair           | Aging; longevity; oxidative stress                                                                   | (Gangestad et al., 2010; González et al., 2020; Pontzer et al., 2015; Ziomkiewicz et al., 2016) |
| Alpha amylase                    | Saliva      | Humans  | Digestion            | Fetal growth; stress response                                                                         | (Broche et al., 2019; Giesbrecht et al., 2015; Petullo et al., 2016)             |
| Cortisol                         | Feces       | Humans  | Stress; energy regulation | Aggression; competitive response; developmental plasticity; energy allocation; energy balance; growth rate; immune response; life history trade-offs; stress; stress response | (Emery Thompson et al., 2010; Flinn & England, 1997; Hinde et al., 2015; Nepomnaschy et al., 2006; Urlacher et al., 2018b; Von Rueden et al., 2014) |
| C-peptide                        | Urine       | Humans  | Energy regulation    | Aging; energy balance; fecundity; hydration                                                           | (Ellison & Valeggia, 2003; Emery Thompson et al., 2012; Higham, Girard-Buttoz, et al., 2011; Reiches et al., 2014; Sherry & Ellison, 2007b; Valeggia & Ellison, 2009a) |
| C-reactive protein (CRP)          | DBS         | Humans  | Immunity             | Energy allocation; inflammation; life history evolution                                               | (Blackwell et al., 2010; Clancy et al., 2013; Lynn et al., 2020; McDade et al., 2008; Thompson et al., 2019; Urlacher, Ellison, et al., 2018) |
| Creatinine                       | Urine       | Humans  | Cellular metabolism  | Muscle mass; energy allocation                                                                       | (Alvarado et al., 2015; Emery Thompson et al., 2016)                               |
| Dehydroepiandrosterone sulfate (DHEAS) | Hair, Saliva, Urine | Humans  | Energy regulation    | Adrenarche; developmental plasticity                                                                 | (Behringer et al., 2012; Helfrecht et al., 2018; Keestra et al., 2021)            |
| Epstein-Barr virus antibody (EBV-Ab) | DBS         | Humans  | Immunity             | Energy allocation; cell-mediated immunity; life history evolution                                      | Eick et al., 2016; Urlacher, Ellison, et al., 2018                               |
| Estradiol                        | DBS         | Humans  | Reproduction         | Energy allocation; fecundity; life history trade-offs; menarche; ovarian function; stress             | (Jasienska et al., 2005; Núñez-de la Mora et al., 2007b; Thompson & Lampl, 2013; Valeggia, 2007; Worthman & Stallings, 1997) |
| Immunoglobulin E (IgE)           | DBS         | Humans  | Immunity             | Adaptive immunity; energy allocation; helminth defense; life history evolution                        | (Blackwell et al., 2010; Cepon-Robins et al., 2021; Urlacher et al., 2021b; Urlacher, Ellison, et al., 2018) |
| Immunoglobulin G (IgG)           | DBS         | Humans  | Immunity             | Adaptive immunity; energy allocation; life history evolution                                          | (Urlacher et al., 2019b; Urlacher et al., 2021a; Urlacher, Ellison, et al., 2018) |
| Ketones                          | Urine       | Humans  | Metabolism           | Energy balance; fat metabolism                                                                       | (Knott, 1998; Naumenko et al., 2020)                                              |
| MIB                      | Sample type | Species | Physiological system | Investigated trait/evolutionary topic                                                                 | Example studies                                                                 |
|--------------------------|-------------|---------|-----------------------|------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Leptin                   | DBS         | Humans  | Energy regulation     | Energy allocation; developmental plasticity; immunity                                               | (Miller et al., 2006; Sharrock et al., 2008)                                           |
| Luteinizing hormone      | DBS, Urine  | Humans  | Reproduction          | Energy allocation; reproductive investment                                                            | (Trumble et al., 2010; Worthman & Stallings, 1997)                                        |
| Neopterin                | Feces, Urine| NHPs    | Immunity              | Disease detection; food competition; innate immunity; mate competition                               | (Behringer et al., 2017b; Heistermann & Higham, 2015; Higham et al., 2015; Sacco et al., 2020) |
| Oxytocin                 | Milk, Saliva,Urine | Humans, NHPs | Neurobehavior | Aggression; cooperation; gonadal function; pair-bonding; parental investment | (Crockford et al., 2013; De Dreu & Kret, 2016; Wittig et al., 2014) |
| Progesterone             | Feces, Saliva, Urine | Humans, NHPs | Reproduction | Aging; energy allocation; Fecundity; ovarian function; life history; menarche; stress response | (Clancy et al., 2013; Ellison et al., 1986; Jasieniska & Ellison, 1998; Núñez-de la Mora et al., 2007a; Valeggia & Ellison, 2004; Vitzthum et al., 2002) |
| Prolactin                | DBS, Urine  | Humans  | Reproduction          | Milk production; Paternal care                                                                        | (Gettler et al., 2015; Worthman & Stallings, 1997; Ziegler et al., 2004)                   |
| Secretory immunoglobulin A (sIgA) | Milk, Saliva | Humans | Immune          | Immunocompetence; offspring immune programming                                                        | (Klein et al., 2018; Lynn et al., 2020; Miller & McConnell, 2015)                           |
| Telomere length          | DBS, Saliva, Skin | Humans, NHPs | Cellular proliferation | Aging; energy allocation; longevity; stress response                                                  | (Eisenberg et al., 2012; Goldman et al., 2018; Rej et al., 2020; Rej et al., 2021; Steinert et al., 2002) |
| Testosterone (T)         | DBS, Feces, Hair, Saliva, Urine | Humans, NHPs | Energy regulation; Reproduction | Aggression; Developmental plasticity; Energy allocation; fecundity; immune activation; male reproduction; mate competition; reproductive success; stress response | (Alvarado et al., 2019; Bibiescas, 1996; Ellison & Panter-Brick, 1996; Emery Thompson, et al., 2012; Gettler et al., 2011b; Kuzawa et al., 2009; Muehlenbein & Watts, 2010; Muller et al., 2009; Trumble et al., 2010) |
| Triiodothyronine (T3)    | Feces, Urine | NHPs    | Energy regulation; Thermoregulation | Energy allocation; energy balance; thermoregulation                                                   | (Chen et al., 2021; Cristóbal-Azkarate et al., 2016; Dias et al., 2017; Gesquiere et al., 2018; Thompson et al., 2017) |

Note: Selected examples (non-comprehensive list). An extended list that includes additional MIBs and individual study entries, species names, and other details is provided in Table S1.
time and energy (i.e., calories) to competing demands across the life course in a manner that maximizes fitness (Charnov, 1991; Stearns, 1992). This simple yet powerful framework – predicting trade-offs between key physiological life tasks as a function of age, environment, and other factors – has been used to shed light on human patterns of growth (Bogin et al., 2007; Leigh, 2001; Urlacher, Ellison, et al., 2018; Urlacher & Kramer, 2018), reproduction (Bribiescas, 2010; Ellison et al., 1993; Emery Thompson et al., 2007; Valeg gia & Ellison, 2009b), immune function (Blackwell et al., 2011; Cepon-Robins et al., 2019; Gurven et al., 2009; McDade, 2003; Muehlenbein & Bribiescas, 2005), physical activity (Caldwell, 2016; Lieberman et al., 2021; Shattuck & Muehlenbein, 2015), aging (Crimmins & Finch, 2006; Gurven et al., 2008; Hawkes, 2003; Jones, 2011; Kaplan et al., 2000; Ziomkiewicz et al., 2016), and other traits.

Two variables that are central to the life history theory approach for understanding phenotypic variation and evolution are energy condition (i.e., energy availability or balance) and energy allocation (i.e., the distribution of energy investment between competing life tasks). Accordingly, these variables have received considerable attention in HEB, with researchers finding success in developing and utilizing MIBs for both. This application of MIBs provides opportunities to objectively assess energetic parameters in field settings. The use of metabolic hormones as MIBs in this area also facilitates direct investigation of the physiological pathways linking environmental stimuli with phenotypic variation (Bribiescas & Ellison, 2008), providing novel opportunity to test mechanistic hypotheses and to increase the sensitivity of life history analysis. Here, we describe several common MIBs of energy condition and allocation in HEB and overview their current application.

2.1.1 | C-peptide

C-peptide is widely applied in HEB as a MIB of energy balance. The hormone insulin, owing to its role directing glucose homeostasis and the synthesis/break-down of energy reserves, is a primary regulator of metabolism and a sensitive marker of energy balance when measured at physiological baseline. C-peptide is a pancreatic byproduct of insulin production that is generated in equimolar fashion to insulin itself. Importantly—and in contrast to insulin—C-peptide is largely inert in circulation and has a relatively long half-life, meaning that circulating concentrations of C-peptide reflect daily insulin production better than circulating insulin itself (Kruszynska et al., 1987). C-peptide, but not insulin, is also excreted intact in urine at a rate directly proportional to production (Kruszynska et al., 1987). For these reasons, urinary C-peptide serves as a reliable MIB of insulin production and, thus, energy balance (Emery Thompson, 2017; Sherry & Ellison, 2007a).

The practicalities of C-peptide analysis in urine samples collected in field settings have been thoroughly described (Emery Thompson & Knott, 2008; Higham, Girard-Buttoz, et al., 2011). Although 24-h urine sampling is recommended, opportunistic collection of urine also provides reliable C-peptide estimates of energy balance in research contexts, particularly when utilizing multi-day sampling. Lab analysis is typically done with enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) and can be performed with conventional urine samples (possessing good long-term stability of C-peptide when continuously frozen) or urine dried and stored on filter paper (Emery Thompson & Knott, 2008; Higham, Girard-Buttoz, et al., 2011; Reiches et al., 2014). A key limitation of C-peptide analysis is that available assays lack standardization, making it particularly difficult to compare data across assays and labs (Little et al., 2008).

The use of urinary C-peptide as a MIB of energy balance has been validated in the lab and field among humans (see above) and a range of comparative wild and captive NHPs, including chimpanzees (Pan troglodytes: Emery Thompson et al., 2009; Sherry & Ellison, 2007a), bonobos (Pan paniscus: Deschner et al., 2008), and several other apes and monkeys (Emery Thompson & Knott, 2008; Für bauer et al., 2020; Girard-Buttoz et al., 2011; Grueter et al., 2014a; Harris et al., 2010; Sacco et al., 2021; Sherry & Ellison, 2007a). Diverse questions have been addressed. A strength, however, has been research relating to female reproductive ecology (Ellison, 2003a) and the study of reproductive variation using energetic models that link environmental conditions with life history trade-offs.

C-peptide has proven a valuable tool in female reproductive ecology in large part because insulin itself acts both directly and indirectly (via gonadotropins) to regulate ovarian steroid hormone production (Greisen et al., 2001). Thus, C-peptide reflects a physiological link between environment and reproductive function/fecundity (Sherry & Ellison, 2007a). The earliest work utilizing C-peptide as an MIB in this framework investigated the drivers of variation in human reproductive rate. In a series of analyses by Valeg gia and Ellison among the Indigenous Qom of Argentina (Ellison & Valeg gia, 2003; Valeg gia & Ellison, 2001; Valeg gia & Ellison, 2004; Valeg gia & Ellison, 2009b), urinary C-peptide tracked across postpartum intervals was used to demonstrate that maternal energy balance declines sharply with birth and the initiation of lactation, recovers to pre-lactation levels.
gradually as moderated by maternal resource availability, and is closely tied to ovarian steroid hormone (estradiol and progesterone) production and the resumption of ovulation. In sum, this work used C-peptide to demonstrate the role of energy balance in the presumed adaptive regulation of fecundity and birth spacing among humans.

Supporting comparative evidence linking urinary C-peptide levels with reproductive function is now also available from wild chimpanzees (Emery Thompson et al., 2012), chacma baboons (Papio ursinus; Fürtbauer et al., 2020), and howler monkeys (Alouatta palliata: Rangel-Negrin et al., 2021). The role of resource availability in moderating rapid recovery of postpartum C-peptide levels and ovulation among humans (Valeggia & Ellison, 2004), for example, has now also been demonstrated among chimpanzees living with varying food resources (Emery Thompson et al., 2012). Highlighting the importance of variation in energy expenditure—as well as energy intake—in this energy balance pathway, the dynamics of postpartum urinary C-peptide levels have also been found to map onto the dynamic costs of milk production and infant transport and vigilance activities among NHPs (Emery Thompson et al., 2012; Rangel-Negrin et al., 2021). The interesting finding that C-peptide levels are greatest during gestation among humans and many NHPs (Fürtbauer et al., 2020; Rangel-Negrin et al., 2021) has led to the hypothesis that primates have evolved a conservative pregnancy metabolic strategy to prepare for the heightened challenges of lactation (Dufour & Sauther, 2002; Fürtbauer et al., 2020). More research is needed in this area to understand the energetic trade-offs that underlie this potential adaptation, with preliminary work investigating urinary C-peptide among Sanje mangabeys (Cercocebus sanjei) indicating the likely importance of trade-offs involving immune activity (McCabe et al., 2016).

2.1.2 | Cortisol

Likely no MIB has received more attention in HEB than cortisol. While its biomarker value is wide, cortisol is often utilized as a MIB of both energy condition and energy allocation toward critical life tasks. Cortisol is a glucocorticoid steroid hormone produced by the cortex as the primary end product of the hypothalamic–pituitary–adrenal axis (Dickerson & Kemeny, 2004; Sapolsky et al., 2000a). Triggered routinely for release by a range of physical and psychosocial “stresors”, including low energy intake and physical activity, cortisol's primary physiological role is to maintain energy homeostasis. To accomplish this, cortisol stimulates catabolic metabolism (i.e., converts energy stores into usable energy) and downregulates a number of costly biological systems, including reproduction, immunity, and growth (Gunnar & Quevedo, 2007). The sensitivity of cortisol production to energetic stress makes it a potential biomarker of energy condition/balance. At the same time, the regulatory effects of cortisol on multiple key life tasks make it a useful biomarker of energy allocation in the study of life history trade-offs.

Researchers in HEB measure cortisol as a MIB in many sample types, including saliva, urine, hair, feces, and milk. Commercial ELISA and RIA kits are typically used for lab assessment. Detailed reviews of the nuances and limitations of cortisol measurement are provided elsewhere (Cook, 2012; Nicolson, 2008; Saxbe, 2008). Here, two major factors warrant mention. First, cortisol has a strong pulsatile and diurnal pattern of secretion among humans and likely most other primates (Adam & Kumari, 2009; Gröschl et al., 2003). Second, and relatedly, sampling strategy (e.g., saliva vs. hair, 24-h urine vs. single-point urine) can have a large impact on the timescale of cortisol production ultimately measured. Point measures of salivary cortisol, for example, are particularly susceptible to the diurnal and pulsatile nature of cortisol secretion and are therefore unlikely to reflect daily cortisol production. The physiological implications of acute cortisol activity differ from those of chronic activity (Do Yup Lee & Choi, 2015, see Developmental Plasticity below), and it is therefore critical that appropriate sampling approaches are selected. Diverse cortisol sampling approaches in HEB make comparisons across studies particularly challenging (Anestis, 2010; Anestis & Bribiescas, 2004).

Cortisol is routinely applied in HEB as a MIB of energy condition, specifically as a MIB of negative energy balance or energy “stress”. This use is derived from extensive experimental evidence from humans (Nieuwenhuizen & Rutters, 2008) and has been supported with comparative evidence from NHPs demonstrating that cortisol in feces, urine, and hair is elevated in contexts of low energy availability, including poor habitat quality (Busch and Hayward, 2009; Jaimez et al., 2012; Rangel-Negrin et al., 2009) and seasonal food scarcity (Gesquiere et al., 2008; Pride, 2005). As expected, the impact of low energy availability on cortisol appears to be greatest among those already at risk for negative energy balance, such as lactating females (Emery Thompson, 2009; Foerster et al., 2012). It is important to note that while the above studies and others provide a strong basis for the use of cortisol as a MIB of energy condition, factors unrelated to energetic stress, such as psychosocial stress, may also influence cortisol production and introduce error into energetic analyses (Emery Thompson, 2017; Flinn, 2010).

To test life history hypotheses, HEB researchers also use cortisol as a MIB of energy allocation. This work
capitalizes on the integrated regulatory role of cortisol across major physiological systems. Under conditions of energetic stress, the evolved function of cortisol is seemingly to prioritize fueling essential life tasks (i.e., those that are critical for survival or most immediate to fitness) at the cost of diverting energy away from tasks that are relatively low priority (Bribiescas, 2010; Charmandari et al., 2005; Ellison, 2017). Thus, cortisol is viewed as a mediator of energetic trade-offs that respond to environmental energy cues. Using cortisol as a MIB under this life history framework, researchers have identified presumed adaptive negative relationships between cortisol and human growth (Nyberg et al., 2010, see Developmental Plasticity below), reproduction (Foerster et al., 2012; Lodge et al., 2013; Valeggia & Ellison, 2009b), immune activity (Hoffman et al., 2011; Muehlenbein, 2006), and other physiological tasks. Notably, fecal cortisol among ring-tail lemurs (Lemur catta: Pride, 2005) and hair cortisol among gray mouse lemurs (Microcebus murinus: Rakotoniaina et al., 2017) have also been shown to predict mortality rate, providing comparative evidence that trade-offs mediated by cortisol are not always enough to ensure survival. As noted by Beehner and Bergman in their excellent review (2017), studies directly testing relationships between cortisol and clear outcomes of fitness are rare among primates, preventing strong conclusions about the adaptive significance of observed energy allocation trade-offs in HEB.

2.1.3 Triiodothyronine (T3)

The thyroid hormone triiodothyronine (T3) has also received attention in HEB as a MIB of energy condition and energy allocation directed to basal metabolic tasks, specifically thermoregulation. Circulating T3 is secreted along with its less biologically active precursor thyroxine (T4) by the action of the hypothalamic–pituitary-thyroid axis. Following additional conversion of T4 to T3 in many peripheral tissues, T3 acts at the cellular level to upregulate ATP production, ultimately increasing cellular metabolic rates and thermogenesis (Danforth & Burger, 1989). Given this direct stimulatory action on many tissues throughout the body (e.g., adipose, hepatic, skeletal muscle), as well as indirect insulin sensitivity-promoting effects (López et al., 2010), T3 levels broadly reflect basal metabolic rate (BMR) among mammals (Leonard et al., 2005) and energy availability and energy balance among humans (López et al., 2013). The regulatory actions of T3 on numerous physiological functions also make it an attractive biomarker of energy allocation, primarily toward thermoregulation (Behringer et al., 2018; Leonard et al., 2005; Mantzouratou et al., 2022).

Traditional assessment of T3 relies on venipuncture blood samples. However, T3 measurement is also validated in urine and feces among humans (Burke et al., 1972; Yoshida et al., 1980) and NHPs (Gesquiere et al., 2018; Schaeds et al., 2016; Wasser et al., 2010). Lab measurement is typically performed using ELISA or RIA. Circulating T3 mirrors cortisol’s dynamic diurnal rhythm (Nicoloff et al., 1970). As such, T3 concentrations in urine and feces are often poorly correlated with blood levels (Burke & Shakespear, 1976; Shakespear & Burke, 1976) and reflect longer-term T3 production (Behringer et al., 2018). Importantly, urinary and fecal T3 measures must account for the presence of both bound and unbound forms of T3 as well as various T3 metabolites that may differ in their physiological significance (Burke & Shakespear, 1976). For these reasons, urinary and fecal T3 assessment must be approached cautiously.

Researchers in HEB capitalize on T3’s role as a regulator of BMR to use T3 as a MIB of energy allocation to basal metabolism in life history analyses. Fecal T3 is regularly measured to investigate energetic response to limited environmental food availability, with the positive relationship between T3 and energy availability/consumption thought to reflect adaptive regulation of basal metabolism to avoid overspending during periods of energy shortage (Cristóbal-Azkarate et al., 2016; Gesquiere et al., 2018; Schaeds et al., 2016; Wasser et al., 2010).

Researchers in HEB have also used T3 to investigate energy allocation to more specific physiological tasks. Thermoregulation has received the most attention in this area, building on work (using predominantly venipuncture samples) indicating that population variation in human metabolic heat production can be explained as T3-mediated adaptation to environmental temperature and cold stress (Cepon et al., 2011; Leonard et al., 2002; Leonard et al., 2005; Levine et al., 1995; Roberts, 1952). Using fecal T3 data, supporting comparative evidence is now available from several species of NHPs (Chen et al., 2021; Gesquiere et al., 2018; Thompson et al., 2017). Interestingly, this research and other work with NHPs (Dias et al., 2021) also demonstrates a positive relationship between fecal T3 levels and male mating effort, a finding that provides comparative evidence for influence of T3 on energy allocation specifically to male reproduction.

2.1.4 Testosterone (T)

Testosterone has been widely used in HEB as a MIB of energy allocation, specifically energy investment in male reproduction. An androgenic steroid hormone, T is
produced in males in the testes as the final product of the hypothalamic-pituitary-testicular axis. Its primary function is anabolic in driving the development and sustainment of male sex characteristics, including spermatogenesis and the synthesis of metabolically costly skeletal muscle tissue (Nieschlag et al., 2012). However, T has broad metabolic functions that also involve the regulation of growth, immune activity, behavior, and other physiological systems. Testosterone's regulatory actions, coupled with its wide observed variation in concentration between species, populations, and individuals (Bribiescas, 2010; Ellison et al., 2002; Schurmeyer & Nieschlag, 1982), make it a key biomarker of energy allocation to male reproduction.

Testosterone can be measured in many sample types, and researchers in HEB currently utilize T as a MIB predominantly in saliva, urine, hair, feces, and milk. This research spans several decades and is being performed among diverse groups of humans and comparative NHPs. The measurement of T is typically performed using commercial ELISA or RIA, but not without limitations. Similar to cortisol, T concentration is labile in circulation, is highly dependent on sample type, and is subject to diurnal, pulsatile, and other biological rhythms (for complete discussion of these issues, see Trost & Mulhall, 2016; van Anders et al., 2014). Importantly, T exists in bound and unbound forms at different levels in different sample types (Keevil et al., 2016) and appears to be particularly susceptible to environmental/behavioral modulation of the relationship between physiological production and clearance (Cadoux-Hudson et al., 1985), influencing measurement. The analytical assessment of T is plagued by a relatively high degree of between-assay and between-lab variation (Rosner et al., 2007). For these reasons, particular care must be taken when assessing T, particularly when making comparisons across studies (Anestis, 2010).

In contrast to several other energetic MIBs common in HEB, T is not a reliable indicator of energy condition. Studies of diet among humans living in non-industrialized populations (e.g., Alvarado et al., 2019; Ellison & Panter-Brick, 1996) and among wild chimpanzees (Muller & Wrangham, 2005) and orangutans (Kott, 1999) demonstrate that T in saliva, urine, and hair is not consistently related to BMI and that short- to intermediate periods of low energy availability have no detectable impact on T. It is generally only with complete fasting that notable decreases in T have been reliably detected among humans (Klibanski et al., 1981; Röjdmark, 1987; Trumble et al., 2010). The impact of regular physical activity on T also appears to be marginal, such that seasonal increases in everyday physical activity are not associated with changes in salivary T among humans (Bentley et al., 1993; Ellison & Panter-Brick, 1996; although see evidence for some changes in diurnal rhythms in Vitzthum et al., 2009). Collectively, this work has led to the conclusion that typical acute and seasonal variation in energy availability and balance has little impact on T (Emery Thompson, 2017; Muehlenbein & Bribiescas, 2010).

The value of T as a MIB of energy allocation to male reproductive effort is much more established. While sperm production itself accounts for <1% of BMR among mammals (Elia, 1992), T also drives male reproductive effort directed toward mate acquisition, including behavioral characteristics that can be energetically costly (e.g., competitiveness, aggressiveness, territoriality, libido) and skeletal muscle mass that is expensive to synthesize and maintain. These costs are substantial. Among male NHPs, it has been estimated that the lifetime energetic costs associated with T's somatic effects alone are equivalent to the direct lifetime reproductive costs experienced by females (i.e., energy invested in pregnancy, lactation, etc., Key & Ross, 1999). The mate-acquisition behaviors promoted by T also appear to incur substantial energy costs for many primates (Alberts et al., 1996; Eberle & Kappeler, 2004; Muller & Wrangham, 2004c). The energetic cost of male reproduction is clearly considerable, and it is regulated by T (Bribiescas, 2001; Muller, 2017). This recognition is applied widely in HEB to test life history theory predictions.

Work in HEB has shown that salivary T among adult males from human subsistence populations in Africa (e.g., Christiansen, 1991; Ellison et al., 1989; Gray et al., 2007), South America (e.g., Beall et al., 1992; Bribiescas, 1996; Trumble et al., 2012), Asia (e.g., Ellison et al., 2002; Ellison & Panter-Brick, 1996), and Oceania (e.g., Campbell, 1994), while exhibiting between-group variation (Alvarado et al., 2019; Ellison et al., 2002), are consistently lower than those of men living in industrialized populations. Wild male chimpanzees similarly have lower urinary T than their captive counterparts (Muller & Wrangham, 2005). A wide body of research is investigating the specific environmental factors that underlie such broad differences in reproductive energy allocation and their potential adaptive significance. In addition to the possible effect of chronic energy limitation during ontogeny in establishing low lifetime T (see Developmental Plasticity below, Bribiescas, 2010), energetic trade-offs involving metabolic tasks in direct competition with immediate reproduction appear likely.

Research using T as a MIB in HEB has provided considerable evidence for trade-offs between reproduction and immune activity (see reviews in Muehlenbein & Bribiescas, 2005; Muehlenbein et al., 2017). However, T's impact on immunity appears to be complex (i.e., regulating specific aspects of immunity unevenly
and adult phenotype onto predicted later life environmental conditions, ultimately increasing fitness (Gluckman et al., 2005). Defining developmental pathways, such as PARs, can improve understanding of variation in phenotype and fitness (Lu et al., 2019) as well as help to identify the underpinnings of individual trajectories of lifetime health outcomes (McDade et al., 2007b).

Many MIB approaches are suited for data collection with individuals of all ages and developmental stages. As such, MIBs have been used to investigate developmental plasticity among a wide range of human groups and comparative NHP species (Behringer et al., 2014; Emery Thompson et al., 2012; Gesquiere et al., 2005; Tkaczyński et al., 2020). This work spans several active areas of HEB research on developmental plasticity, including studies on response to early life adversity, life history flexibility, and biosocial pathways of variation in development and health. Problematically, most HEB research on developmental plasticity remains limited to retrospective investigation of prior ontogenetic responses using data collected from adults. Relatively few studies have utilized longitudinal MIB approaches to track trait specific variation in association with early life conditions. This dearth of longitudinal data poses considerable challenges for identifying adaptation and fitness effects.

Here, we overview several MIBs used in HEB to investigate developmental plasticity and highlight their key research applications and potential for expanded use.

2.2 Developmental plasticity

Human evolutionary biologists and anthropologists have been interested in how early life conditions shape human development and lifelong phenotype for well over a century (Boas, 1912; Chisholm et al., 1993; Ellis et al., 2009; West-Eberhard, 2003b). Within HEB, developmental plasticity—the phenomenon by which a single genotype can produce distinct phenotypes in response to variation in early environmental conditions (Lea et al., 2017)—remains a key concept and a focus of research on the evolutionary mechanisms underlying human diversity.

Current adaptive theories on developmental plasticity in HEB posit that individuals respond developmentally to variation in experienced conditions in order to facilitate either immediate fitness benefits (e.g., survival, “immediate adaptive responses”) or to facilitate lifetime fitness benefits (e.g., future reproduction, “predictive adaptive responses [PARs]”) (Ellis & Del Giudice, 2019; Gluckman et al., 2005; Kuzawa & Bragg, 2013; Lea et al., 2017). In the case of PARs, it is suggested that evolved biological mechanisms allow individuals to respond to early environmental cues (within genetically-defined limits and norms of reaction) in order to target their development and adult phenotype onto predicted later life environmental conditions, ultimately increasing fitness (Gluckman et al., 2005). Defining developmental pathways, such as PARs, can improve understanding of variation in phenotype and fitness (Lu et al., 2019) as well as help to identify the underpinnings of individual trajectories of lifetime health outcomes (McDade et al., 2007b).

2.2.1 Cortisol

Cortisol is again used in HEB as a MIB of experienced “stress” and a mediator of adaptive phenotypic response in investigations of developmental plasticity. Cortisol is released as a result of various stressors, including energetic challenges and adverse social situations (Lu et al., 2019). Its regulatory roles can be applied to understand developmental plasticity, whereby cortisol production in high-stress early environments may serve to influence development and adult phenotype in response signals of a challenging lifetime environment.

Several studies in HEB have assessed cortisol levels among pregnant women or in breast milk as a MIB of experienced maternal stress. In the adaptive framework, maternal cortisol is hypothesized to transmit signals of environmental quality to the fetus, resulting in calibrated developmental plasticity that enhances immediate survival and long-term fitness. Support for this hypothesis in HEB has been found in humans demonstrating that elevated maternal evening salivary cortisol levels are associated with reduced fetal growth rate (Thayer et al., 2012). Similar comparative evidence is available from NHPs
(Bardi & Huffman, 2006; Berghänel et al., 2016; Mustoe et al., 2014). In breast milk, cortisol concentrations may reflect the maternal environment and their own experienced life history (Hinde et al., 2015). A growing body of evidence among humans (Grey et al., 2013; Hahn-Holbrook et al., 2016) and NHPs (Bernstein & Hinde, 2016; Dettmer et al., 2018; Hinde et al., 2015; Hinde & Milligan, 2011; Petruollo et al., 2019; Sullivan et al., 2011) indicates that milk cortisol levels influence offspring development as predicted by evolutionary theory. This work supports cortisol’s key role as a signal of environmental stress to offspring in the postnatal period, supporting adaptive PAR theory in which offspring respond early in life to environmental signals to optimize lifetime phenotype and fitness.

Cortisol is also used as a MIB of an individual’s own experienced stress in HEB developmental plasticity research. Among children in the subsistence Tsimane population of Bolivia, for example, elevated morning and evening salivary cortisol levels—their levels driven by immunological stress—were found to be predictive of current child growth outcomes (Nyberg et al., 2012). Retrospectively, many studies in HEB using saliva, urine, feces, and hair samples have also identified relationships linking early life adversity among humans (e.g., low socioeconomic status, childhood trauma, familial stress) with cortisol patterns in adulthood (Desantis et al., 2015; Kim et al., 2020; Zhang et al., 2019). Similar relationships among wild chimpanzees (Tkaczynski et al., 2020) provide comparative evidence that socioecological challenges during early life (e.g., feeding competition, poor maternal care, parent-offspring conflict) alter cortisol levels across development. Chronic stress exposure among humans and NHPs results, somewhat unintuitively, in diminished rather than elevated cortisol production, including lower overall cortisol levels and blunted reactivity and diurnal rhythms (Ellis & Del Giudice, 2019; Ponzi et al., 2020; Sapolsky et al., 2000b). This downregulation in salivary cortisol production has been demonstrated at the population level among subsistence human groups subject to chronic stressors early in life (Nyberg, 2012; Urlacher et al., 2018c). Cortisol suppression in the presence of chronic stress may function as an adaptive mechanism to maintain physiological homeostasis and prevent deleterious outcomes of hypercortisolism, such as persistent wasting and immunosuppression (Nyberg, 2012; Urlacher et al., 2018c). This hypothesis warrants further investigation.

2.2.2 | Progesterone

Developmental plasticity provides an important framework for understanding variation in lifetime reproductive phenotypes (Jasienska et al., 2017). In HEB, progesterone has often been used as an MIB of reproductive effort in developmental plasticity analysis. Progesterone is an ovarian pregnane steroid hormone that plays important roles in female sexual development, ovarian function, pregnancy, lactation, and other reproductive activities (Jasienska et al., 2017; Nunez-de-la-Mora & Bentley, 2008). It can be measured using commercial ELISA and RIA kits directly in saliva, hair, and DBS and indirectly via metabolites in urine (e.g., pregnanediol glucuronide [PgD]) and feces (e.g., 5α-pregnan-3α-ol-20-one [5-P-3OH]). A full review of progesterone measurement is provided elsewhere (Gildner, 2021a). Progesterone production varies predictably across the menstrual cycle, with levels increasing during the luteal phase to prepare the uterus for implantation and support conception (Taraborrelli, 2015). Progesterone concentration is also influenced acutely by a number of other factors, including diet and exercise (Ellison, 2003b). As such, repeat measures of progesterone on multiple days across the menstrual cycle and over multiple cycles is typically needed to reliably document habitual patterns, particularly when measuring the hormone in saliva (Jasienska & Jasienski, 2008).

Adult progesterone levels appear to be influenced by early life environmental conditions (Jasienska et al., 2017), and researchers in HEB have utilized progesterone extensively as an MIB to investigate developmental plasticity in human female reproductive investment (Ellison et al., 1986; Lipson & Ellison, 1992; Núñez-de-la Mora et al., 2007a; Vitzthum et al., 2002). From a life history perspective, progesterone adjustments can be adaptive in regulating reproductive investment and probability of conception as a function of environmental cues on energy availability and social support or extrinsic mortality (Ellison, 1996; Ellison, 2003b). This approach has proven useful for understanding variation in reproductive function in women across several populations, including among rural Polish farmers (Jasienska & Ellison, 2004), Bangladeshi migrants (Núñez-de la Mora et al., 2007a), and rural Bolivian Aymara women (Vitzthum et al., 2004). Among Bangladeshi migrants to London, for example, it was shown that women who migrated during childhood have higher levels of average luteal salivary progesterone than women who never migrated and continue to reside in Bangladesh (Núñez-de-la Mora et al., 2007a). Illustrating the importance of timing on developmental plasticity in ovarian function, those women who migrated earliest in development displayed the highest salivary progesterone levels and experienced the earliest age of menarche (Núñez-de la Mora et al., 2007a). Supporting evidence is available from several species of NHPs, demonstrating the impact of a broader range of early-life socioecological variables (e.g., maternal dominance rank, natal dispersal) on adult...
progesterone levels and variation in female reproductive function (Gesquiere et al., 2005; Onyango et al., 2013; Sousa & Ziegler, 1998; Xia et al., 2018).

2.2.3 | Testosterone (T)

As described above, T is a commonly utilized MIB of male reproductive effort in HEB. Within the context of developmental plasticity, studies in HEB have utilized T to understand how socioecological conditions experienced during early life also influence male reproductive investment across the lifespan. This body of research is more limited than for cortisol or progesterone but is still worth mention. Most of the work on this topic exists for humans (Alvergne et al., 2008; Magid et al., 2018; Thompson & Lampl, 2013; Trumble et al., 2013), and supports the hypothesis that variation in energetic factors (i.e., nutritional intake, pathogen load) during development adaptively influences adult reproductive investment (i.e., timing of reproductive maturity, adult reproductive function). For example, it has been shown that Bangladeshi men who migrated to London during childhood display higher levels of waking and evening salivary T levels and experience earlier pubertal onset than men who completed their childhood in Sylhet (Magid et al., 2018). Importantly, this pathway may help to more broadly explain the lower T levels documented among subsistence populations relative to industrialized references (see Energetics and Life History Variation/Evolution). Comparative NHP data on this topic is lacking, and more research is needed to identify the specific socioecological variables that drive lifetime variation in T and male reproductive function.

2.3 | Social status and dominance

Research on social dynamics has a rich history in HEB, including the study of human and NHP social stratification and hierarchies (Hawley, 1999; Sapolsky, 2005). Minimally invasive biomarkers are widely used to operationalize and test evolutionary hypotheses in this area (Harris & Schorpp, 2018). Two related traits have received a particularly large amount of attention: social status among humans and social dominance among NHPs. Human social status is defined as relative access to resources and is a function of how members of a social group evaluate one another based on prestige or influence (Henrich & Gil-White, 2001). It is linked to differences in skill, respect, and power (Berger et al., 1972; Berger et al., 1980; Desjardins et al., 2015). Comparatively, in NHPs, social dominance is more broadly defined as a relative measure of “coexistence” among members of a social group, where coexistence is determined by resource acquisition, reproduction, and health (Dewsbury, 1982; Drews, 1993; Majolo et al., 2012; Sapolsky, 2005). Social dominance is usually represented in terms of social rank and can be applied to individual and dyadic relationships through dominance hierarchies that mediate interactions such as conflict and competition (Newton-Fisher, 2017).

Given the importance of social status and dominance as traits for understanding social dynamics and related evolutionary processes, researchers in HEB have developed several MIB approaches to investigate them. Here, we highlight three of the most studied MIBs in HEB social status and dominance research.

2.3.1 | C-peptide

As outlined above, C-peptide is widely used in HEB as a MIB of human and NHP energy balance. This utility has proven useful for research investigating the energetic correlates and evolutionary selective pressures surrounding social dominance among many NHPs (Deschner et al., 2008; Emery Thompson et al., 2009; Grueter et al., 2014b; Higham et al., 2011; Sacco et al., 2021; Surbeck et al., 2015). Unfortunately, similar applications with social status among humans, while suggested (Valeggia & Ellison, 2009b), have not been directly performed.

Available C-peptide MIB research among NHPs consistently indicates that obtaining and maintaining social dominance comes with a considerable energetic cost. Among adult male chimpanzees, for example, high-ranking individuals have lower urinary C-peptide levels than lower-ranking individuals across multiple seasons (Emery Thompson et al., 2009). It has been suggested that this apparent energetic cost is related to greater aggression and foraging effort necessary to maintain social dominance (Emery Thompson & Georgiev, 2014). Research among rhesus macaques offers additional insight into the dynamics of social dominance energetics, demonstrating that dominant male urinary C-peptide levels plummet during periods of heightened mating effort and are inversely related to obtained copulation events (Higham et al., 2011). Together, these findings provide insight into the evolution of primate social dynamics and structure, highlighting the role of social competitiveness strategies and dominance energetics in shaping distributions in male reproductive success.

C-peptide is also used as a MIB of energy balance to investigate the evolutionary links between primate social relationships and party size. The evolution and nature of
grouping patterns in primates is hypothesized to relate, in part, to access to food (Anderson et al., 2002). Providing support for this hypothesis, longitudinal research among bonobos—characterized by fission–fusion social dynamics—has demonstrated a positive association between mean monthly urinary C-peptide levels and mean party size, indicating larger group formation and reduced hostility in times of greater energy abundance (Surbeck et al., 2015). Interestingly, when in large groups, dominant male bonobos have greater, not lower, C-peptide levels than their low-ranking counterparts. This indicates increased contest competition over food, shedding light on the dynamic dominance-energetic relationships underlying the evolution of primate social systems (Emery Thompson et al., 2009; Surbeck et al., 2015). These findings can be applied to understand the evolution of human social systems and indicate the promise of similar application of C-peptide as a MIB in human research.

2.3.2 | Cortisol

Cortisol’s role as a MIB of stress has also been applied in HEB to investigate social dynamics. Specifically, cortisol is used to illuminate relationships between social status/dominance and experienced stress. This has led to better understanding of the evolution of human and NHP social systems and life history strategies.

Cortisol levels among humans are well established to have an inverse relationship with social status (Sherman & Mehta, 2020). In HEB, this relationship has now been demonstrated using a range of cortisol and social status measures among small-scale populations. This work includes research using multi-point salivary cortisol assessment among adult male villagers in Dominica (Decker, 2000) and subsistence forager-horticulturalists in Papua New Guinea (Konečná & Urlacher, 2017), as well as research measuring first-morning urine cortisol among subsistence forager-horticulturalists of all ages in Bolivia (Jaeggi et al., 2021; Von Rueden et al., 2014). Interestingly, no such relationship was found between social status and hair cortisol concentration among relatively egalitarian female hunter-gatherers of Tanzania (Fedurek et al., 2020). The totality of evidence, however, from both industrialized and small-scale populations indicates a typically strong inverse relationship between human social status and cortisol levels. This relationship is widely interpreted to reflect lower physical (i.e., energetic) and/or psychosocial stress among high-status individuals. Within-group distributions of stress have direct developmental plasticity and life history implications relating to lifelong variation in phenotype and fitness (see above).

Comparative data from NHPs indicates that, in contrast to findings for humans, greater social dominance is related to greater cortisol concentrations. This finding is robust and includes studies assessing urinary and fecal cortisol levels among chimpanzees (Emery Thompson et al., 2020; Muller & Wrangham, 2004b; Preis et al., 2019), bonobos (Surbeck et al., 2012; Wobber et al., 2010), capuchins (Schoof et al., 2012; Schrock et al., 2019), baboons (Weingrill et al., 2004), macaques (Barrett et al., 2002; Higham et al., 2013; Qin et al., 2013; Vandeleest et al., 2020; Zhang et al., 2018), and platyrhines (Bales et al., 2006). The observation of positive relationships between cortisol and dominance rank aligns with that for C-peptide among NHPs and points to the importance of physical/energetic challenges experienced by high-ranking individuals. The nature of the difference in the social status/dominance-cortisol relationship between humans and NHPs remains unclear, but it is likely driven by differences in the relative strictness of social hierarchies, rank stability, and environmental resource abundance (Sapolsky, 2005). Regardless, these MIB findings indicate something unique about the evolution of human social dynamics and social structure.

2.3.3 | Testosterone (T)

The use of T as a MIB has also led to novel evolutionary insights into the links between social status/dominance and male behavioral energetics and life history. Among its many androgenic actions, T is known to regulate striving and aggressive dominance behaviors (Casto & Edwards, 2016; Kordsmeyer et al., 2019). Researchers in HEB have capitalized on this role, and applications of T as a MIB within related evolutionary social behavioral contexts can now be found among humans (see review in Booth et al., 2006; Muehlenbein & Bribiescas, 2005) and a long list of comparative NHPs (Emery Thompson et al., 2012; Marty et al., 2015; Mendonça-Furtado et al., 2014; Muehlenbein et al., 2004; Muehlenbein & Watts, 2010; Muller & Wrangham, 2004a; Ross & French, 2011; Schoof et al., 2014; Wobber et al., 2010).

Positive associations between status/dominance and male T concentrations have been observed using salivary samples among humans (Booth et al., 2006) as well urine samples among chimpanzees and gorillas (Muller & Wrangham, 2004a; Robbins & Czekala, 1997) and fecal samples among bearded capuchins (Mendonça-Furtado et al., 2014). These findings support the view of primate T as a “competition hormone” that calibrates status-
enhancing motivations through aggressive and competitive behaviors (Mazur & Booth, 1997), ultimately improving reproductive success and fitness (Bribiescas, 2001). Notably, however, the positive relationship between social status/dominance and T has not been consistently identified across human and NHP studies (e.g., Kordsmeyer et al., 2019). Deviation from this pattern has typically been explained with the “Challenge Hypothesis” (Wingfield, 2017; Wingfield et al., 1990). The Challenge Hypothesis suggests that natural selection has often favored the evolution of a physiological system in which testosterone levels are flexible and increase only when most critical for fitness (e.g., during male–male competition to improve performance and survivability), thereby avoiding the many deleterious consequences of chronically high T levels (e.g., wasting, immunosuppression). Support for the Challenge Hypothesis has been provided using salivary measures of T among humans in small-scale populations (Trumble et al., 2012) as well as using urinary and fecal measures of T among wild chimpanzees (Muller & Wrangham, 2004a) and several other NHPs (Cavigelli & Pereira, 2000; Girard-Buttoz et al., 2015). Collectively, this research points to complex relationships linking T with dominance behaviors and the evolution of social dynamics and hierarchies among humans and their primate relatives. More work is needed to directly investigate the fitness implications of these relationships.

3 | UNDER-INVESTIGATED MIB APPLICATIONS IN HEB

3.1 | MIBs of linear growth

Linear growth is often a focus of research in HEB. At the species and population levels, human and NHP patterns of growth are studied as windows into environmental conditions, life history evolution, and the selective forces that have shaped body size and the dynamics of ontogeny (Bogin, 1999; Cheverud et al., 1992; Kuo & Chen, 2017; Leigh, 2001; Tanner et al., 1990; Urlacher, Blackwell, et al., 2016). At the level of the individual, linear growth variation is often used to investigate developmental plasticity and evolved norms of reaction (Bogin et al., 2007; Hochberg, 2011; Urlacher, Liebert, et al., 2016). The now well-established relationship between human growth and later life chronic disease risk (Dewey & Begum, 2011) also makes linear growth a focal point for understanding the developmental origins of health and disease within the HEB subfield of evolutionary medicine (Berger et al., 2021).

Despite a long-standing focus on linear growth in HEB, the field has been slow to adopt new methods of growth assessment to complement traditional anthropometry. This has limited research opportunities. Anthropometric assessment of linear growth (e.g., in stature among humans and comparative crown-rump length among NHPs) is characterized by a relatively high degree of measurement error, requires multi-point data collection to document current growth – typically over a period of several months or longer – and is often prohibitively difficult or invasive to perform among NHPs (Mattison & Vaughan, 2017). A small number of HEB studies among both humans and NHPs have explored the possibility of measuring serum insulin-like growth factor 1 (IGF-1) as a biomarker for linear growth (see review in Bernstein, 2017). However, the invasiveness of this approach (currently requiring venipuncture) and the activity of IGF-1 on multiple biological systems other than linear growth has limited its application.

Several MIBs of linear growth—increasingly common in other fields—offer promise for overcoming methodological limitations in HEB. These MIBs are objective, single-point, and are being developed predominantly using urine and DBS samples that are familiar to researchers. Typically, they use commercially available ELISA or RIA kits. Anthropometry will remain a critical tool for assessing growth in HEB. However, MIBs of linear growth, if properly validated, offer alternative or complementary measures that may be useful in specific contexts. Here, we highlight three analytes that hold promise as MIBs of linear growth.

3.1.1 | C-terminal telopeptide (CTx)

C-terminal telopeptide is an established marker of bone resorption and turnover. It is a small peptide fragment that is cleaved from collagen type 1 and released into circulation in a manner proportional to collagen degradation by osteoclasts (Rosen et al., 2000). Due to high sensitivity and specificity to osteoclast activity, CTx in blood has been widely used clinically and in research as a measure of human bone resorption and turnover rate (see review in Herrmann & Seibel, 2008). It has also been used for this purpose among long-tailed macaques (e.g., Stroup et al., 2001; Wu et al., 2021). The small size of CTx allows it to be cleared by the kidneys, and it can be reliably measured in urine using a commercial ELISA kit. The dynamics of CTx and the nuances of its measurement in urine are well documented. Measurement is typically done using first morning void urine samples in fasted state in order to control for effects of food intake (Holst et al., 2007) and time of day (Bjarnason et al., 2002). A large reference for CTx values in healthy Austrian children age 0–18 years old (Rauzenzauner et al., 2007) provides a means for calculating CTx
percentiles and performing sensitive statistical analysis. Levels of CTx mirror linear growth rates throughout childhood and during the adolescent growth spurt (Matsukura et al., 2003; Rauchenzauner et al., 2007; Szulc et al., 2000), demonstrating the utility of CTx as a MIB of growth.

### 3.1.2 Osteocalcin

Osteocalcin is an established marker of bone formation. A major non-collagenous matrix protein synthesized primarily by osteoblasts, osteocalcin in its carboxylated form has high affinity for hydroxyapatite and directly binds bone. As such, circulating osteocalcin levels have been shown to be directly proportional to osteoblast activity and rate of bone formation (Booth et al., 2013; Zoch et al., 2016). The short half-life of osteocalcin and its diurnal rhythm of production (Shetty, 2016) render it most indicative of short-term bone activity. Clinically, osteocalcin levels in blood are often used to monitor bone formation in metabolic diseases such as osteoporosis (Chen et al., 2000; Kuo & Chen, 2017). Importantly, serum osteocalcin is also currently used as a biomarker of linear growth, often in the context of disorders such as growth hormone deficiency and growth faltering (Zoch et al., 2016) but also in relation to rapid bone deposition and growth during adolescence (Lee et al., 2000). These studies demonstrate the close positive relationship between circulating osteocalcin and current linear growth. As with CTx, a large reference for serum osteocalcin values is available from healthy Austrian children age 0–18 years old (Rauchenzauner et al., 2007). A recent validation has established that osteocalcin can also be reliably measured in DBS samples using a modified protocol with a commercially available ELISA kit (Eick et al., 2019). Collectively, this work supports exploring TRACP-5b for application as a MIB in HEB growth-related research.

### 3.1.3 Tartrate resistant acid phosphate (TRACP-5b)

Tartrate resistant acid phosphate is another promising MIB of bone resorption and linear growth. Secreted by osteoclasts, TRACP-5b is a small polypeptide chain. Its biological function is unknown, yet its circulating levels correlate closely with osteoclast number. As such, it is widely used as a blood marker of bone resorption and turnover (Haleen et al., 2006; Jancikila & Yam, 2009). Its concentration in serum has been shown to correlate positively with children's linear growth (Lin et al., 2016). Similar to CTx and osteocalcin, a large reference for serum TRACP-5b values is available from healthy Austrian children age 0–18 years old (Rauchenzauner et al., 2007). One measurement and potential biomarker advantage over CTx and osteocalcin is that TRACP-5b is more stable in circulation, with minimal diurnal variability or response to feeding (Jancikila & Yam, 2009). As for osteocalcin, a recent validation has established that TRACP-5b can be reliably measured in DBS samples using a modified protocol with a commercially available ELISA kit (Eick et al., 2019). Collectively, this work supports exploring TRACP-5b for application as a MIB in HEB growth-related research.

### 3.2 MIBs of gut function

Gut function has been an important topic of study in HEB for several decades. The Expensive Tissue Hypothesis (Aiello & Wheeler, 1995), for example, was formative in proposing an energetic link between gut size and encephalization in the evolution of primates. More recently, researchers in HEB have embraced the study of the gut microbiome to investigate variation in human and comparative NHP metabolism, life history, and health (Amato et al., 2016; Bennett et al., 2016; Clayton et al., 2018; Tung et al., 2015). Despite this sustained research focus on the gut, little remains known about variation in some of the most basic aspects of human and NHP gut function, including gut absorptive capacity and gut permeability. Gut absorptive capacity (i.e., ability to extract nutrients from ingested food) has direct implications for foraging efficiency and, ultimately, energy availability. Gut permeability (i.e., integrity of the gut barrier) has implications for host-pathogen interactions and, ultimately, energy expenditure via ability to prevent ingested pathogens from entering circulation and triggering costly immune responses.

Evidence from fields outside of HEB suggests that variation in gut absorptive capacity and permeability are substantial among humans (Damm-Machado et al., 2017; Dutta et al., 2019; Li & Atkinson, 2015) as well as NHPs (Bethune et al., 2008; Garg et al., 2015; Zhang et al., 2020). One primary source of variation globally among humans appears to be environmental enteric dysfunction (EED)—an acquired condition characterized by microbial translocation across the small intestine and reduced absorptive capacity—that is common in contexts of high pathogen exposure (Kosek, 2017; Marie et al., 2018; Mbuya & Humphrey, 2016; Prendergast et al., 2014).

Documenting human and NHP variation in gut absorptive capacity and permeability and investigating the ecological drivers of such variation is a critical step...
toward understanding the evolution of digestion, metabolism, and life history as well as the mechanisms regulating health and disease. The use of MIBs is a promising approach for accomplishing this task. Several MIBs of gut absorption and permeability have already been developed in the sciences. Here, we highlight three of the most promising MIBs for application in HEB.

3.2.1 | Claudins

Claudins are a family of proteins that are established biomarkers of gut absorptive capacity, a position relating to their role as key regulators of tight junction ion selectivity in the intestinal epithelial barrier (Guerrant et al., 2016b; Harper et al., 2018). Two claudins to receive particular attention by researchers are Claudin-15 and Claudin-2. These are used clinically in detecting intestinal damage and early stages of development of diseases such as necrotizing enterocolitis (Blackwood et al., 2017) and inflammatory bowel disease (Thuijls et al., 2010). Circulating levels of Claudin-15 in humans have been shown to closely reflect intestinal barrier absorptive function as determined using gold-standard jejunal biopsy (Guerrant et al., 2016b; Günzel & Yu, 2013). Recently, urine concentrations of both Claudin-15 and Claudin-2—measured using commercial ELISA—have similarly been shown to exhibit positive relationships with children’s gut absorptive capacity assessed using dual sugar test (Guerrant et al., 2016b) and jejunal biopsy (Blackwood et al., 2017), respectively. This initial validation work is promising. However, additional research is needed to determine the usefulness of urinary claudins as MIBs of gut absorptive capacity in HEB.

3.2.2 | Zonulin

Zonulin is a promising biomarker of gut permeability. A haptoglobin protein that induces the breakdown of epithelial tight junctions, zonulin acts to reversibly increase intestinal permeability (Fasano, 2011; Fasano, 2012). Clinically, it has been indicated in the pathogenesis of several chronic inflammatory conditions, including celiac disease and type 1 diabetes (Ciccia et al., 2017; Fasano, 2011; Fasano, 2020; Hendy et al., 2017; Kümé et al., 2017). Further supporting its role as a biomarker of gut permeability, circulating zonulin is consistently elevated among children suffering from or presumed to be suffering from EED (Guerrant et al., 2016a; Tickell et al., 2019; Zambruni et al., 2019). A commercial ELISA kit has been developed for measuring zonulin in fecal samples, and fecal zonulin has also been shown to correlate well with dual sugar test measures of gut permeability (Damms-Machado et al., 2017; Seethaler et al., 2021). Zonulin is also a promising candidate for measurement in DBS, but additional validation work is needed.

3.2.3 | Endotoxin-core antibodies (EndoCAb)

Endotoxin-core antibodies (EndoCAb) are an additional promising biomarker of gut permeability. The production of EndoCAb and its concentration in circulation increases as a degree of intestinal permeability and associated translocation of bacterial products across the epithelial barrier into the bloodstream (Brenchley & Douek, 2012). Given this strong, albeit indirect, relationship with gut permeability, EndoCAb measured by venipuncture blood sampling has been successfully used as a biomarker of gut permeability in numerous studies examining EED and other intestinal conditions among humans (Campbell et al., 2003; Mondal et al., 2012; Mwape et al., 2017; Uddin et al., 2021). Importantly, EndoCAb measurement using a commercial ELISA kit has been preliminarily validated for minimally invasive DBS, with a strong reported correlation (r = 0.965) between matched DBS and plasma values (Hoke et al., 2018). This study, although limited by a small sample size (N = 18), provides initial support for the application of EndoCAb as a MIB of gut permeability in HEB.

3.3 | Epigenetic MIBs of developmental plasticity

Epigenetic research continues to gain interest in HEB. Epigenetic modifications are heritable changes in gene expression which occur via biochemical mechanisms (i.e., DNA methylation, histone modification, noncoding RNA’s) that do not alter the gene sequence itself but that can have large impacts on phenotype (Mulligan, 2016; Non & Thayer, 2019). Researchers in HEB have taken interest in epigenetic modifications due to characteristics such as sensitivity to environmental experiences (i.e., nutritional intake, psychosocial stress, toxicant exposure, racial disparities) (Kuzawa & Sweet, 2009; Thayer & Kuzawa, 2011) and the ability to influence phenotypic variation across the life course. At the individual, population, and species levels, HEB epigenetics research is being used to investigate evolutionary questions on life history, developmental plasticity, and the developmental origins of health and disease (Lea et al., 2017; Mulligan, 2016; Thayer & Kuzawa, 2011).
Problematically, existing epigenetics research in HEB among contemporary human populations has largely been performed using invasive venipuncture blood samples. Recent validations of minimally invasive sample types provide opportunity to expand this work using epigenetic MIBs. Here, we highlight two common epigenetic markers that offer promise for application as MIBs of developmental plasticity in HEB.

3.3.1 | DNA methylation patterns

DNA methylation is a well characterized epigenetic mechanism that involves covalent binding of a methyl group to DNA on cytosines, primarily in CpG sites, inhibiting binding of transcription factors otherwise needed for gene expression (Langie et al., 2017). It is a primary epigenetic pathway altering gene regulation in mammals, and patterns of methylation can help explain basic developmental processes as well as lifetime phenotypic responses to environmental stimuli (Burdge & Lillycrop, 2010). Patterns of methylation can be identified globally or in a more targeted manner using PCR and other technologies with small quantities of DNA (Singer, 2019). Research in HEB using invasive venipuncture blood sampling has demonstrated the utility of DNA methylation assessment for addressing developmental plasticity and evolutionary questions, including the adaptive nature of the impact of human early life social environments (McDade et al., 2017) and resource availability (McDade et al., 2019) on adult inflammatory immune processes. Comparative work among NHPs has provided additional evidence linking early environmental conditions to adaptive plasticity in several adult behavioral/physiological phenotypic traits (Anderson et al., 2021; Lea et al., 2016; Tung et al., 2012). This work is promising. However, recent advancements in the ability to assess methylation patterns in minimally invasive sample types such as feces (Belshaw et al., 2004), urine (Jatkoe et al., 2015), DBS (Hollegaard et al., 2013), saliva (Matthews et al., 2013), and buccal cells (Claire Mulot et al., 2004) provides opportunity to much more broadly and systematically utilize MIB approaches of DNA methylation in HEB.

3.3.2 | Telomere length

Telomere length is epigenetically regulated via DNA methylation and represents another promising MIB of developmental plasticity and variation in biological aging. Structurally, telomeres are repetitive DNA sequences that comprise the cap-like protein structures found at the ends of chromosomes (Blackburn, 1991; Eisenberg, 2011). The length of telomeres is reduced over the lifespan with successive DNA replication, increasing opportunity for damage to the genome and accelerating biological senescence (Eisenberg, 2011). However, telomeres are susceptible to DNA methylation (Levis et al., 1985) and evidence suggests that this modification functions to reduce telomere shortening throughout life (García-Cao et al., 2002; García-Cao et al., 2004; Gonzalo et al., 2005; Gonzalo et al., 2006). Telomere length is thus used as an epigenetic marker of biological aging. Evolutionarily, telomere length may serve as a biomarker of variation in energy allocated to cellular maintenance (e.g., anti-aging) activity across the life course (Eisenberg, 2011). Recent work has validated the measurement of telomere length in minimally invasive saliva, DBS, and buccal cell samples (Goldman et al., 2018; Rej et al., 2021; Stout et al., 2017), and researchers in HEB have preliminarily started to adopt the use of telomere length as an MIB. This initial research has led to the identification of negative effects of psychosocial stress (Rej et al., 2020) and adverse childhood experiences (Puterman et al., 2016) on human telomere length, suggesting more rapid biological aging with early life experienced adversity. This work supports the proposed adaptive nature of variation in telomere length and warrants additional MIB work in HEB.

4 | FUTURE DIRECTIONS FOR MIB RESEARCH IN HEB

Despite decades of progress and many successes, limitations remain in HEB research using MIBs. Many of these limitations have been noted above for specific situations but require more general discussion. Here, we point to some of the most promising future directions for advancing MIB research in HEB (Figure 1).

4.1 | Expand the MIB toolkit

4.1.1 | New MIBs

There will always be opportunity to develop new MIBs to expand the MIB toolkit and HEB’s range of investigation. Many promising MIBs are already validated and in use in other fields, awaiting application to address evolutionary questions (see examples for growth, etc., above). These MIBs often require relatively little or no additional validation work and are particularly attractive for new research directions. In other cases, biomarkers already established with invasive sample types warrant
development and validation as MIBs. Prospective MIBs must be evaluated carefully, with consideration of many potential factors limiting their application in new sample types (see validation discussion below; Lindau & McDade, 2008).

4.1.2 | Multi-analyte approaches

Approaches that use multiple MIBs in combination hold promise in HEB, most obviously by facilitating investigation of novel relationships between two or more traits but also by providing information across different timescales and by improving ability to disentangle traits with multiple environmental inputs (e.g., the source of “stress”). The current use of multi-analyte approaches is more common in HEB research among humans than NHPs. However, several recent examples demonstrate the value of multi-MIB approaches for investigating comparative NHP topics ranging from developmental plasticity of the stress response (salivary cortisol and alpha-amylase: Petruetto et al., 2016) to the behavioral and environmental sources of energy imbalance (urinary C-peptide and fecal T3: Rangel-Negrin et al., 2021). The ability to utilize multiple MIBs is becoming more practical with the development of multi-analyte laboratory techniques that minimize sample waste (see below).

4.1.3 | New minimally invasive sample types

Researchers in HEB are well positioned to be leaders in expanding the range of minimally invasive sample types used in MIB assessment. There are several promising biospecimen types to consider. Nail clippings, for example, are now established in several fields as viable for the measurement of hormones and other analytes (Jaramillo Ortiz et al., 2021), most notably cortisol (Fischer et al., 2020). Recent work in HEB has shown the promise of this approach, demonstrating that fingernail cortisol levels among forager children of the Congo Basin are inversely correlated with measures of experienced fathering quality (Gettler et al., 2021). Sample types such as skin (Wang & Maibach, 2011), breath (Das & Pal, 2020), and undried finger-prick whole blood (Blackwell et al., 2021) offer additional promise for MIB development in specific situations and with specific analytes.

4.1.4 | New analytical measurement methods

Most MIB research in HEB continues to use single-analyte immunoassays (ELISA and RIA) for biomarker laboratory measurement. These methods are established and relatively affordable and approachable (Grange et al., 2014). Increasingly, however, researchers are turning to alternative methods to provide greater analytical sensitivity or to measure multiple analytes at once. Bead-based immunoassays (i.e., multiplex assays) and flow cytometry have been used to measure panels of immunological biomarkers among humans and NHPs using venous blood (Giavedoni, 2005) and, in more limited instances, using minimally invasive sample types (for a recent example with DBS, see McDade et al., 2021). Liquid chromatography mass spectrometry (LCMS)—
validated for measuring a range of biomarkers in urine and feces (Murtagh et al., 2013)—has also been applied in HEB for sensitive MIB assessment (Vogel et al., 2012; Weltring et al., 2012).

The primary factors limiting wider use of multi-analyte measurement approaches in HEB are the complexity and costs involved in purchasing, maintaining, and operating needed equipment. These challenges will not easily be overcome and disproportionately impact researchers based in the low- and middle-income countries where much HEB research continues to be performed. A focus of the field should therefore be on utilizing analytical approaches that not only perform well but are accessible (e.g., widely available, low cost, easy to use). In this regard, point-of-care (POC) measurement technologies hold promise. Some POC technologies are already regularly used to measure MIBs in HEB research, including dipstick urine test strips to detect the presence of ketones and metabolic stress (e.g., Knott, 1998; Muller & Wrangham, 2005; Naumenko et al., 2020) and portable photometric and electrochemical devices that use finger-prick blood to measure circulating hemoglobin, glucose, and blood lipids (e.g., DeLouize et al., 2022; Levy et al., 2018; Liebert et al., 2013; Raichlen et al., 2017). However, as discussed by Gildner et al. in their recent review of POC technology (Gildner et al., 2021), barriers to POC application remain, including a general lack of device validations performed under typical HEB field conditions.

4.2 | Improve MIB reliability

4.2.1 | Robust validations for each MIB (for each sample type, for each species)

Many reviews have been published on the methodological issues associated with MIB research and the importance of proper validations for ensuring reliability (Buchanan & Goldsmith, 2004; Saxbe, 2008; Touma & Palme, 2005; Trost & Mulhall, 2016), including reviews specific to HEB work with humans and NHPs (e.g., Anestis, 2010; Behringer & Deschner, 2017; Gray et al., 2017; Higham, 2016; McDade, 2013). In general, proper validations should be performed at both the analytical/chemical level—Is the measurement of the analyte accurate and reliable?—and the biological/physiological level—Does the analyte accurately and reliably reflect the trait of interest?—for each MIB, for each sample type, for each species.

Robust MIB validations are not currently the standard in HEB, although there are many excellent examples (e.g., Behringer et al., 2017a; Eick et al., 2016; Emery Thompson & Knott, 2008; Higham et al., 2020; McDade et al., 2004). High quality analytical/chemical validations must report a range of MIB measurement characteristics, including sensitivity, specificity, linearity/parallelism, and biomarker stability. Biological/physiological validations should also be performed with each new sample type proposed to demonstrate that a MIB does indeed reflect the trait of interest. Similarly, the application of MIBs to new comparative primate species—possessing potential differences in biomarker structures and sensitivities—must be justified. Additional underexplored issues to consider in MIB validations include variation in the relationship between biomarker production and clearance rates and the biological significance of carrier proteins and metabolite conjugation that may moderate biomarker activity and relationships with phenotype.

4.2.2 | Standardized approaches in the field, lab, and office

There is likely no more effective or efficient way to advance the use of MIBs in HEB than to adopt standardized approaches for collecting, analyzing, and reporting data (Mulligan et al., in press; Poisot et al., 2019). Research groups should freely share and, ideally, publish detailed protocols for data collection and analysis. This information should include the specifics of sample collection, any data collection tools, lab protocols and reference/control information, and code and programs developed for data analysis. This standardization would lead to higher quality research and would improve reliability when comparing MIB findings across studies, research sites, and labs.

4.3 | Better support hypotheses of adaptation

4.3.1 | Complementary measures of energy expenditure

The role of energetics in many HEB studies using MIBs has been established. Energetics are central to MIB research addressing life history questions. Hormones like cortisol, T, and T3, for example, appear to have evolved as physiological messengers to coordinate adaptive patterns of energy expenditure. Including these MIBs in research designs can therefore support the existence of energetic trade-offs between competing life tasks by targeting the pathways of energy regulation that underlie observed correlations of traits (Bribiescas & Ellison, 2008). While such analyses are powerful, this
approach is limited by not actually measuring energy expenditure or accounting for potential differences in daily energy budgets. Recent research demonstrates the usefulness of study designs that pair MIBs with complementary measures of energy expenditure to investigate adaptive patterns of energy use. Research among hunter-gatherers in Tanzania, for example, has combined urine measures of oxidative stress with urine doubly labeled water (DLW) measures of daily total energy expenditure (TEE, kcal/day) to support the existence of fitness-enhancing trade-offs between somatic aging defense and other life tasks (Pontzer et al., 2015). Research among forager-horticulturalists of Ecuador has similarly combined MIBs of immune activity with DLW measures of TEE and respirometry-based (breath) measures of basal metabolism to provide “calorie-level” evidence for adaptive energetic trade-offs between immune activity and childhood growth (Urlacher et al., 2019a; Urlacher et al., 2021a). Studies such as these highlight the promise of combining MIBs and measures of energy expenditure to better support adaptive energetic hypotheses.

4.3.2 | Evidence of functional redundancy

It is well established in evolutionary biology that the regulation of variation in a phenotypic trait (i.e., the regulation of plasticity) is itself a trait that can be selected and adaptive (West-Eberhard, 2003a). Recognizing this, a growing target of HEB research is to demonstrate the fitness-enhancing functions of regulatory physiological pathways, for example those involved in energetic trade-offs (Bribiescas & Ellison, 2008; Flatt & Heyland, 2011; Ricklefs & Wikelski, 2002; Zera & Harshman, 2001). This work is promising, but it often struggles to demonstrate that the regulation of a trait or set of traits is indeed adaptive and not the result of pathology (i.e., breakdown of physiological function) or chance evolution (e.g., random selection, genetic drift). Providing evidence of functional redundancy in regulatory pathways may be helpful in such cases. Functional redundancy supports the adaptive nature of a trait by providing evidence for a specific evolved function and evolved complexity (Williams, 2008). Demonstrating that multiple branches of a single regulatory pathway serve the exact same redundant function—for example the inhibition of child growth by not one but numerous direct and indirect actions of inflammatory immune activity (Cirillo et al., 2017)—increases confidence in identifying regulation that is adaptive and evolved via natural selection. More research in HEB should aim to demonstrate functional redundancy, for example using multi-MIB approaches, to support adaptive hypotheses.

4.3.3 | Longitudinal measures of fitness

As noted by others (Beehner & Bergman, 2017), research in HEB has disproportionately focused on identifying the sources of variation in MIBs rather than on their presumed relationships with fitness. Troublingly few studies in HEB have collected the longitudinal data necessary to reliably document fitness effects for presumed adaptive traits. Identifying lifetime reproductive success should be the goal of this work. Yet, even longitudinally assessed proxies for fitness (e.g., inter-birth interval, offspring survival) are often lacking in evolutionary hypothesis testing. Measuring lifetime fitness outcomes among humans and other primates with slow life histories is inherently difficult owing to the challenges of sustaining long-term research infrastructure and funding. However, a growing number of successful HEB field studies utilizing MIBs offer examples for approaches for compiling the robust longitudinal datasets that will guide future advancements in the field by demonstrating lifetime fitness effects.

5 | CONCLUSIONS

Research in HEB has a rich history using MIBs to document variation in key aspects of human and comparative NHP phenotype. A wide range of MIBs are also being productively applied to operationalize and test evolutionary hypotheses, including those relating to topics such as life history, developmental plasticity, and social dynamics. This work is expansive and was only cursorily covered in this paper. Given the breadth/depth of its current MIB research activity and the nature of its research foundation in both the field and in the lab, HEB is well-positioned to remain a leader in MIB development and application. We have outlined several promising avenues for advancing the field. Apart from continuing to expand the MIB toolkit, researchers should improve trust in MIB reliability and better support hypotheses of adaptation. The use of MIBs is likely to remain a hallmark of HEB for many years to come, and continued progress in this area holds promise for advancing our understanding of what is means to be human.

AUTHOR CONTRIBUTIONS

Samuel S. Urlacher: conceptualization (lead), writing – original draft (lead), writing – review and editing (lead). Elizabeth Y. Kim: conceptualization (equal), writing – original draft (equal), writing – review and editing (equal). Tiffany Luan: conceptualization (equal), writing – original draft (equal), writing – review and editing (supporting). Lauren J. Young: conceptualization (supporting), writing – original draft (supporting), writing –
review and editing (supporting). Brian Adjetey: writing – original draft (supporting), writing – review and editing (supporting).

ACKNOWLEDGMENTS
We thank the other participants of the “Minimally Invasive Biomarkers in Human Population Biology Research: State of the Science and Future Directions” invited poster session at the 2019 American Association of Physical Anthropology annual meeting for their feedback on an earlier version of this paper. We also thank our anonymous reviewers and other members of the Human Evolutionary Biology and Health Lab for their helpful critiques.

CONFLICT OF INTEREST
The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT
Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Uurlacher, S. S., Kim, E. Y., Luan, T., Young, L. J., & Adjetey, B. (2022). Minimally invasive biomarkers in human and non-human primate evolutionary biology: Tools for understanding variation and adaptation. American Journal of Human Biology, 34(11), e23811. https://doi.org/10.1002/ajhb.23811