The myth of the metabolic baseline: how sleep-wake cycles undermine a foundational assumption in organismal biology

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ABSTRACT

Basal and standard metabolic rates (BMR and SMR) are cornerstones of physiological ecology and are assumed to be relatively fixed intrinsic properties of organisms that represent the minimum energy required to sustain life. However, this assumption is conceptually flawed. Many core maintenance processes underlying SMR are temporally partitioned across sleep and wakefulness and are not continuously active. We argue that instead of representing a singular metabolic state, SMR is better defined as a shifting metabolic mosaic where maintenance functions are distributed unevenly across sleep-wake states. SMR measured during wakefulness will mainly represent ion regulation, thermoregulation, sensory processing, and substrate cycling. In contrast, sleep-measured SMR primarily includes processes upregulated during sleep, including protein synthesis, cellular repair, immune activation, and synaptic plasticity. Our models demonstrate that SMR values measured exclusively during wake or sleep consistently over- or underestimate daily maintenance costs depending on the time spent in specific sleep states and when SMR was measured. In addition, treatment or environmental effects on the costs of specific processes may be entirely missed if metabolic measures occur during the wrong sleep-wake state. The temporal partitioning of maintenance processes suggests that, to date, SMR measurements may have confounded true metabolic variation with individual and species-specific differences in sleep architecture, with implications for the estimation of energy budgets, trait heritability, environmental effects on metabolic rate, and metabolic scaling relationships. We propose redefining organismal maintenance costs as a time-integrated profile of metabolic demands, but also suggest that state-specific SMR measurements are appropriate if the sleep-wake measurement period aligns with that of the behavioural, physiological, or ecological context of interest. Moving beyond the fiction of a constant maintenance baseline would provide more refined insights into the bioenergetic foundations of ecological performance and evolutionary constraints.

The Unstable Foundation of Metabolic Rate

Basal and standard metabolic rates (BMR and SMR) are among the most widely measured and applied traits in organismal biology. Both terms refer to the rate of energy throughput by a whole organism for baseline maintenance processes under standardized resting conditions. BMR applies to measurements at thermoneutral temperatures in endotherms, and SMR refers to measurements at any specified temperature in endotherms and ectotherms[1]. The ubiquity of BMR and SMR (hereafter collectively referred to as SMR) in research stems from their scalability across biological levels, from cells to ecosystems, and their integration into foundational ecological theories[2–4]. SMR often correlates with other fundamental organismal traits (e.g. growth rate[5]), and is also used to derive traits such as aerobic scope[6]. These standardized estimates of energy use are fundamental across fields, influencing the study of life-history strategies, responses to environmental change, species interactions, and population dynamics[7–9] .

Standard metabolic measurements implicitly assume a constant baseline energy rate for maintenance processes in resting animals. This conceptualization treats cellular and physiological maintenance as being continuously active with a single, measurable SMR. However, this assumption masks a critical biological reality: different maintenance processes are activated or downregulated, depending on what the animal is doing, with a especially strong divide between waking and sleep states. Indeed, while some biological processes are downregulated during sleep[10], many others are upregulated[11]. As a result, there is not a continuous metabolic baseline, but instead a metabolic mosaic of shifting maintenance energy demands that vary across the sleep-wake cycle based on which processes are active or suppressed. Indeed, a primary hypothesis for sleep's function is an efficient energy reallocation among maintenance functions that is incompatible with wakefulness[12]. Consequently, SMR estimates taken during a single state – such as during sleep – capture only the maintenance costs that are predominant in that state, potentially misestimating both process-specific and whole-animal costs in other states.

Despite its ubiquity across animal taxa, sleep remains an underexplored source of variation in ecological and comparative physiology. Sleep architecture refers to the duration, fragmentation, latency, and distribution of non-rapid eye movement (NREM) and rapid eye movement (REM) sleep states, and variation. Across species, sleep duration and structure correlate with factors influencing SMR, including body size and life-history characteristics[13]. Within species, individuals show consistent differences in sleep architecture, linked to behavioural types and energy budgeting[14]. This variation may drive physiological differences often attributed to intrinsic metabolic traits. Specifically, SMR differences among treatments, individuals, or species, may reflect either: (1) true physiological variance in SMR; (2) variation in sleep-wake architecture; or (3) variation in the sleep-wake state during which SMR was measured – three potential sources of variation that are likely often conflated but must be disentangled for an accurate biological interpretation of SMR (Figure 1). Environmental factors, such as temperature, photoperiod, and habitat structure, affect the time spent in different sleep-wake states[15,16] and the corresponding maintenance costs, therefore further confounding metabolic measurements.

To fully utilize SMR as a meaningful physiological trait, we must recognize that it is not a fixed or static measure, but the sum of multiple maintenance processes that varies over time with sleep-wake state. Here, we synthesize literature on SMR's physiological components and their differential expression across sleep-wake states, to broadly estimate the state-partitioning of maintenance functions. In doing so, we propose a fundamental shift toward redefining maintenance metabolism as a dynamic, state-dependent profile shaped by sleep-wake cycles and suggest a re-evaluation of how metabolic traits are measured, interpreted, and applied.

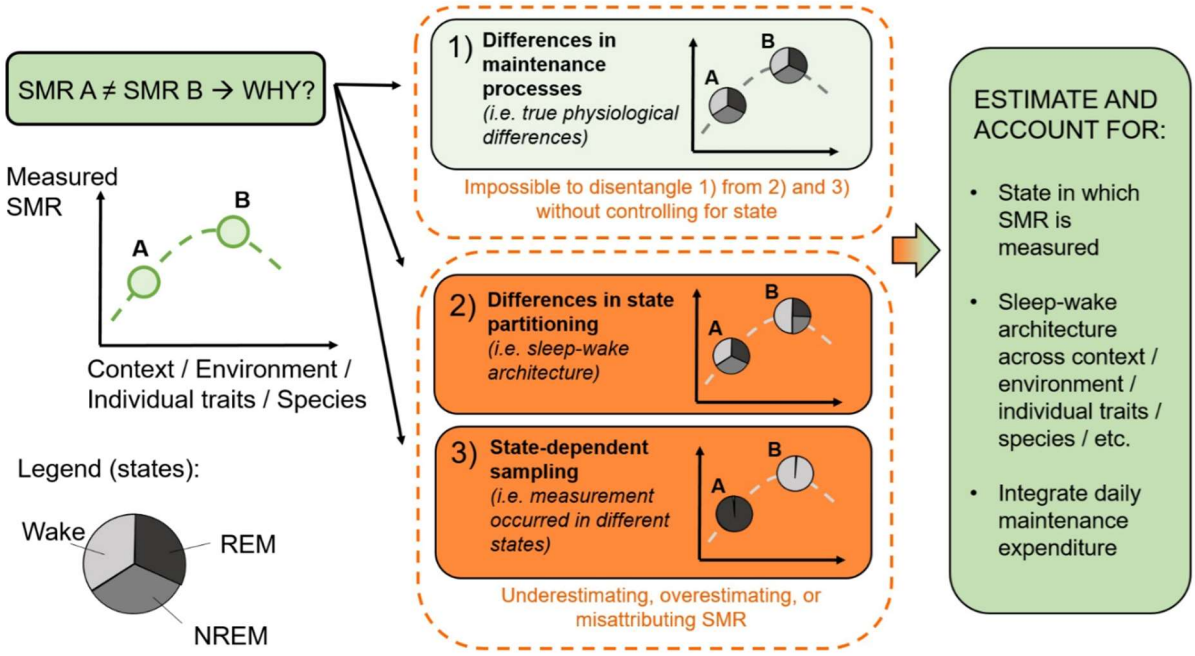

Figure 1. Disentangling physiological variation from state-dependent effects and sampling artefacts. Differences in standard metabolic rate (SMR) between individuals, contexts, or species may reflect real variation in maintenance metabolism (1), but also differences in sleep-wake architecture (2) or the sleep-wake state during which measurements were conducted (3). Without controlling for sleep-wake state, these sources of variation are confounded, causing misestimation of whole-animal SMR and treatment effects on specific maintenance processes. Each pie chart illustrates proportions of time spent in wake, NREM, and REM states, which differ across contexts or individuals and affect SMR estimates. Accurate measurement and interpretation of SMR requires specifying the state in which measurements occur, quantifying sleep-wake architecture, or integrating across sleep-wake states to approximate daily maintenance expenditure.

Why There Is No Static SMR: The Case for State-Dependent Partitioning

To illustrate how state-dependence can influence SMR estimation, we first broke down SMR into its constituent maintenance processes, such as ion gradient maintenance, protein synthesis, and thermoregulation (see Figure 2 and Supplement 1 for the full list of SMR maintenance processes included). We then estimated the proportional contribution of each major maintenance process to overall SMR across sleep-wake states by using available estimates from the literature. However, for many processes, direct quantification of energetic costs across sleep-wake states do not exist. In these cases, we derived informed estimates by combining available data on the relative contribution of each process to total SMR with evidence for how the underlying physiological systems, organs, or cellular mechanisms are up- or down-regulated across different sleep-wake states.

We recognize that this approach necessarily involves inference and that our quantitative estimates should be interpreted cautiously. In addition, the values used here are drawn primarily from studies of humans and other mammals, for which studies are most abundant, and substantial variation likely exists across taxa, individuals, and environmental contexts. However, we are not aiming to provide definitive quantitative estimates for all species and situations, but are instead demonstrating that maintenance processes are unlikely to be equally active across all sleep-wake states and offer a biologically informed heuristic for understanding how overlooking state-partitioning can lead to systematic biases in SMR measurement. While our specific partitioning values lack exactness, the broader biological reality that different maintenance functions are temporally partitioned across sleep and wakefulness is well-established, and the metabolic consequences of this partitioning warrant additional quantitative research focus and consideration in metabolic rate studies.

Below, we highlight the state-dependent partitioning of several key maintenance processes that exemplify different patterns of temporal allocation across sleep-wake states. The partitioning estimates and supporting evidence for the remaining processes are provided in Supplement 1.

Brain Ion Regulation

Maintaining ion gradients across neuronal membranes is one of the most energetically expensive functions in the brain. It has been estimated that ion pumping via Na^+/K^+-ATPase accounts for roughly half of total cortical ATP consumption, particularly under awake conditions[17,18]. Given that the cerebral cortex accounts for approximately 20% of SMR in adult humans[1], this implies that cortical ion-pumping alone accounts for ~10% of whole-body SMR. However, this estimate excludes regions such as the thalamus, basal ganglia, cerebellum, and brainstem, which also maintain high baseline activity and demand for ion transport[19]. To account for these additional brain structures, we conservatively assign 15% of SMR to total brain ion-gradient maintenance. During wake, cortical firing rates increase, driving maximal Na^+/K^+-ATPase activity[17,18]. During NREM, neuron firing rates decline by ~40%[18,20], which should theoretically reduce Na^+/K^+-ATPase activity proportionally. This is supported by metabolic evidence showing 25-44% reductions in brain glucose and oxygen metabolism during deep NREM sleep, with REM sleep showing partial rebound of firing rates[21]. Based on this evidence, we partition overall brain ion regulation activity as 50% during wake, 15% during NREM, and 35% during REM sleep states (Figure 2; Table S1).

Peripheral Ion Regulation

Even at rest, skeletal muscle consumes significant energy to maintain ionic balance. In resting human quadriceps, for example, Na^+/K^+-ATPase and sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) together account for approximately 25% of muscle oxygen use[1]. Given that skeletal muscle comprises ~40% of human body mass, this implies a whole-body contribution to SMR of ~10-12% from peripheral ion regulation. Although peripheral tissues like skeletal muscle and viscera require ionic gradient maintenance continuously, activity is also modulated by postural tone and other factors dependent on sleep-wake cycles, with high tonic muscle activation during waking periods necessitating increased Na^+/K^+-ATPase and SERCA activity[22]. Conversely, muscle tone is reduced during NREM and almost completely absent during REM due to active brainstem inhibition of motoneurons[22–24]. Therefore, energetic demand for peripheral ion regulation is likely to follow a similar pattern to brain ion regulation across sleep-wake states (Figure 2; Table S1).

Protein Synthesis and Cellular Repair

Whole-body protein turnover is a major component of maintenance metabolism, with protein synthesis and degradation together accounting for 18-25% of standard metabolic rate[1]. Wakefulness is associated with basal protein turnover, but sleep – particularly NREM – is the primary period for upregulation of genes involved in protein synthesis and folding[25,26]. Additional support comes from sleep deprivation studies showing that prolonged wake suppresses these pathways, which rebound during recovery sleep[27]. Accordingly, we have partitioned these costs to reflect higher biosynthetic activity during sleep (Figure 2; Table S1).

Thermoregulation

Thermoregulation is a key component of maintenance metabolism in endotherms, typically accounting for approximately 10-15% of SMR under resting conditions at or near thermoneutrality[1]. While this cost primarily reflects active thermoregulatory control mechanisms, sleep strongly modulates these thermoregulatory activities in a state-dependent manner. During wakefulness, thermoregulatory reflexes are fully functional, allowing precise control of core body temperature[28]. In contrast, NREM sleep is associated with a mild suppression of thermoregulatory control, including reductions in core and cortical temperature and decreased responsiveness to thermal challenges[29]. Although heat continues to be produced by basal metabolic processes, the defensive mechanisms that maintain temperature set points are downregulated. During REM sleep, thermoregulatory defenses are almost entirely disengaged or suppressed[11,29], possibly to reallocate resources

to neural processes[30]. Based on this evidence, we allocate 70% of thermoregulatory energy expenditure to wakefulness, 25% to NREM, and 5% to REM.

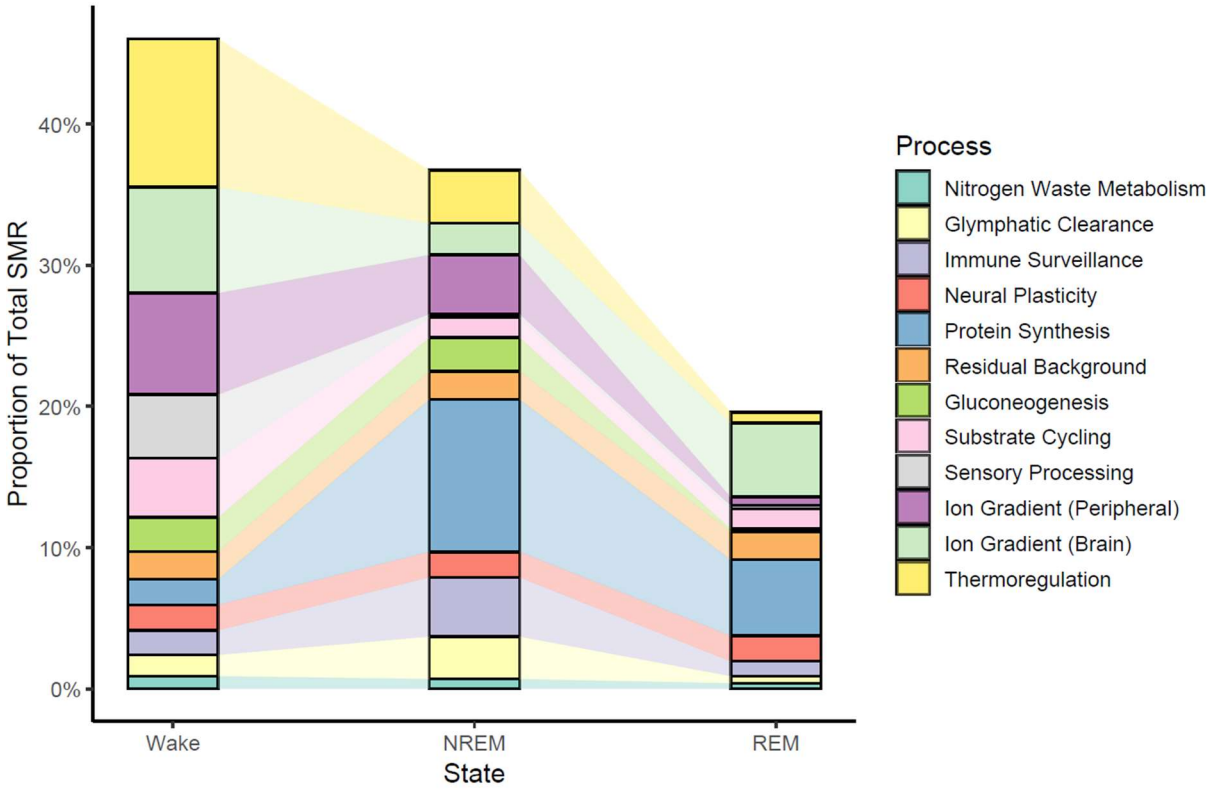

Figure 2. Estimated state-dependent allocation of maintenance costs contributing to standard metabolic rate (SMR). Bars represent the proportion of total SMR attributable to different physiological maintenance processes when measured during wakefulness, non-rapid eye movement (NREM) sleep, or rapid eye movement (REM) sleep, generated from the values in Table S1. Values are scaled such that the total SMR measured across all states sums to 100%.

Overall Analysis

These trends suggest that there is no singular or fixed value for SMR and that true organismal maintenance costs are best represented as an integrated measure of shifting maintenance processes that are differentially expressed across sleep-wake states. Specifically, costly processes such as thermoregulation, ion gradient maintenance, and sensory processing are upregulated during wakefulness. Conversely, protein synthesis, baseline immunity, and neural plasticity are upregulated during sleep, and especially during NREM. As a result, the metabolic profile of each state is likely to differ substantially in both magnitude and composition (Table S1, Figure 2), and so any SMR measurement taken during a single state is likely to capture a biased portion of maintenance costs, either overrepresenting the energetically intensive demands of wakefulness or underrepresenting them during sleep. Importantly, this bias is structured by the organism's sleep architecture, which can vary with context, individual traits, and species.

An important caveat to our analysis is that the baseline SMR values and process contributions underlying Table 1 have been derived from studies that were likely affected by the same sleep-wake biases we discuss here, being measured under uncontrolled or unspecified sleep-wake conditions. As such, they may already reflect state-dependent sampling artefacts instead of true physiological costs. This creates a somewhat circular problem, because we are using potentially biased data to quantify the magnitude of bias in metabolic rate measurements. However, this limitation highlights a key motivation for measurement refinements and the conceptual shift we are proposing. Not only are new methods needed to improve future studies, but they are required to

retrospectively validate (or correct) existing metabolic rate data that may have been affected by unrecognised sleep-wake artefacts.

Consequences of Overlooking State-Dependent Maintenance Costs

The unequal partitioning of maintenance processes across sleep and wakefulness creates two broad problems: (1) Sleep architecture will influence whole-animal SMR estimation and misrepresent maintenance costs (Figure 3, 4); and (2) Measuring SMR during only one state accounts for only a portion of maintenance processes, and so the magnitude of observed effects of a factor or treatment on SMR will vary depending on the maintenance processes that are affected and the state in which SMR is measured (Figure 5). For example, if a wake-predominant process like substrate cycling is upregulated due to a treatment, sleep-only SMR measurements won't accurately reflect this change in maintenance costs.

Estimating error in SMR from state-limited sampling

To examine the first type of error, we developed a model that explored how state-specific SMR measurements diverge from true 24-hour maintenance costs under varying sleep-wake schedules and REM sleep proportions (Figure 3; see Supplement 2 for details). This simulation illustrates the error produced when SMR is estimated solely during either sleep or wakefulness, using partitioning estimates shown in Figure 2 and Table S1. When SMR is measured exclusively during sleep (Figure 3A), estimated values increasingly underestimate the true integrated 24-hour maintenance costs as the proportion of the day normally spent awake increases. On the other hand, if SMR is measured only during wakefulness (Figure 3B), estimates increasingly overestimate true 24-hour maintenance costs as the duration of unmeasured sleep increases. Notably, both types of bias will be exacerbated in individuals or species with a higher proportion of REM sleep, which is associated with particularly low metabolic activity.

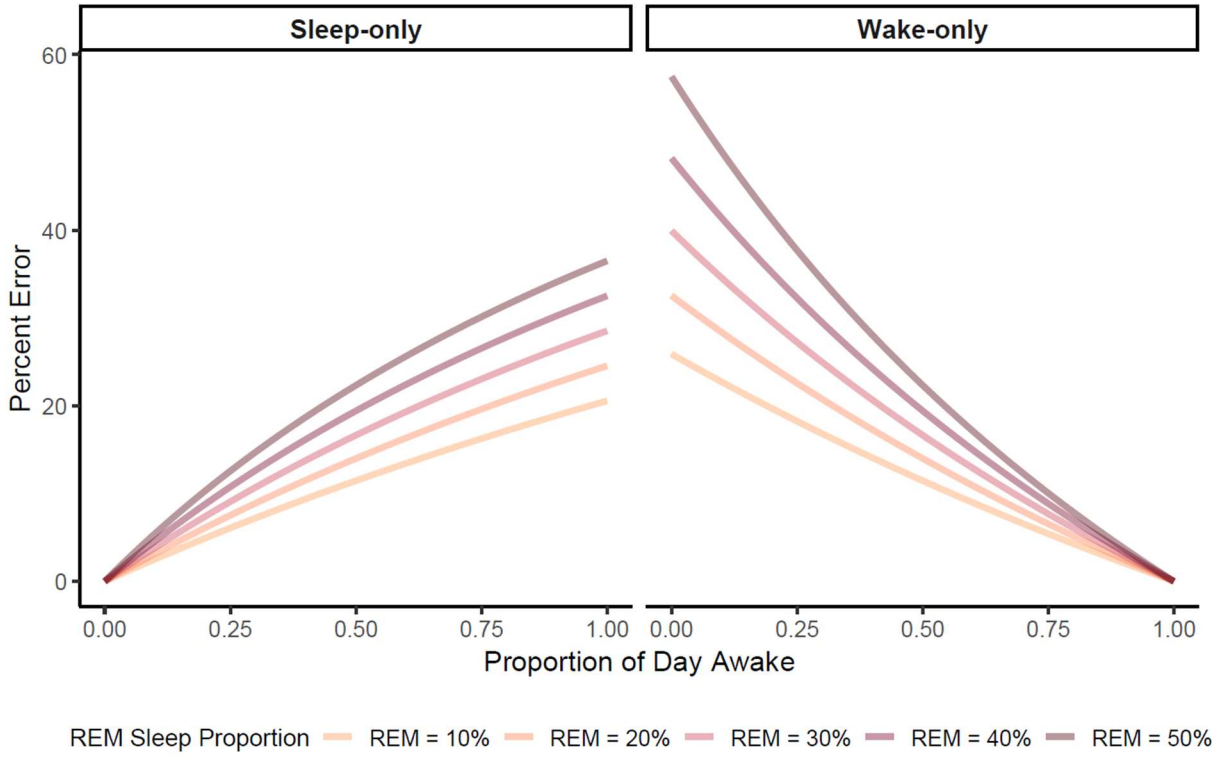

Figure 3. Predicted error in standard metabolic rate estimation when measured exclusively during wake or sleep. Percent error in estimated standard metabolic rate (SMR) is shown as a function of the proportion of the day normally spent awake for a given individual or species, assuming SMR is measured only during sleep (A) or

only during wakefulness (B). Errors are expressed relative to the true integrated 24-hour SMR. The model assumes a fixed contribution of each state to SMR (wake = 44%, NREM = 37%, REM = 19%; Figure 1; Table 1).

Effects of state-dependent partitioning on SMR and aerobic scope estimation

We then developed an individual-based model to examine how variation in sleep architecture may bias estimates of both SMR and aerobic scope (Supplement 2 for details). Specifically, we simulated repeated overnight measurements for a population of individuals differing in their true integrated SMR, total sleep duration, and proportion of REM sleep, allowing us to examine how these factors interact to produce misestimates of SMR. We found that although the proportion of SMR missed during sleep-only sampling remains constant, individuals with higher true SMRs experience larger absolute errors (Figure 3A). Importantly, this error in SMR estimation carries over to affect calculations of aerobic scope (Figure 3B). While MMR was held constant across states, SMR varies due to differing maintenance demands during wakefulness, NREM, and REM sleep. Consequently, using sleep-based SMR measurements to infer aerobic capacity may overestimate the performance capacity achievable during wakefulness. Notably, calculations of aerobic scope provide an example where state-specific SMR values, rather than a 24-hour integrated SMR value, are most appropriate, since the latter would reflect average aerobic capacity across a circadian cycle, whereas state-specific values more accurately represent performance limits relevant to the behavioural or ecological context being studied during a given state (e.g. wakefulness).

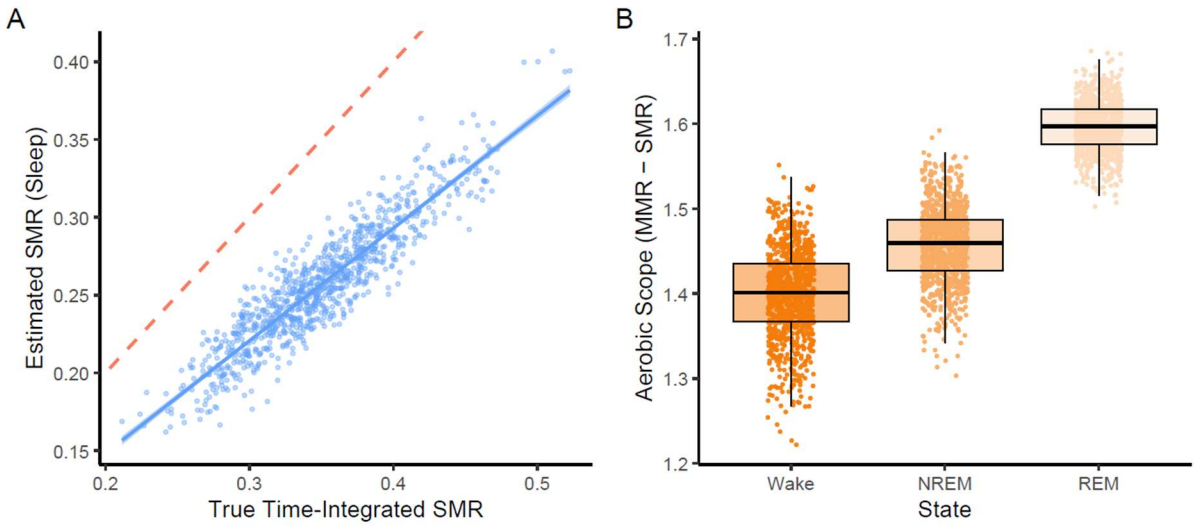

Figure 4. Simulated effects of overnight measurements on estimates of SMR and aerobic scope, and the influence of sleep architecture. (A) Each point represents a simulated diurnal individual (n = 200), measured on each of five nights (five points per individual). The x-axis shows true standard metabolic rate (SMR), defined as the ideally measured and time-integrated average over 24 hours and accounting for partitioned energy use across wake, NREM, and REM states. The y-axis shows the SMR that would be estimated if measured during a fixed 12-hour overnight window. The dashed red line represents points where sleep-estimated SMR equals the true time-integrated SMR. (B) Aerobic scope (MMR-SMR) is shown for each sleep-wake state, based on simulated state-specific SMR values.

Impact of state-restricted SMR measurements on detection of treatment effects

To examine how state-dependent maintenance processes can bias estimates of treatment or environmental effects on SMR estimates, we developed an individual-based model that simulates how SMR and specific process costs are partitioned across sleep-wake states (Supplement 2 for model details). A hypothetical inhibitor treatment was applied in this simulation that reduced costs of protein synthesis by 50% across all sleep-wake

states. Treatment effects were calculated both from the 24-hour integrated SMR estimates, and those based only from measurements during sleep (NREM and REM), while allowing individuals to vary in total sleep duration and the proportion of REM within sleep. Due to the upregulation of protein synthesis during sleep, sleep-only measurements show a greater effect size as compared to the true integrated daily treatment effect (Figure 4A and C), suggesting that apparent among-individual variability in treatment responses may partly reflect differences in sleep architecture, rather than true physiological heterogeneity. For example, a treatment that alters thermoregulatory costs or ion regulation (predominantly active during wakefulness), or protein synthesis (mainly during sleep), could be substantially underestimated or entirely missed if measurements are taken during the wrong state (Figure 4A, B).

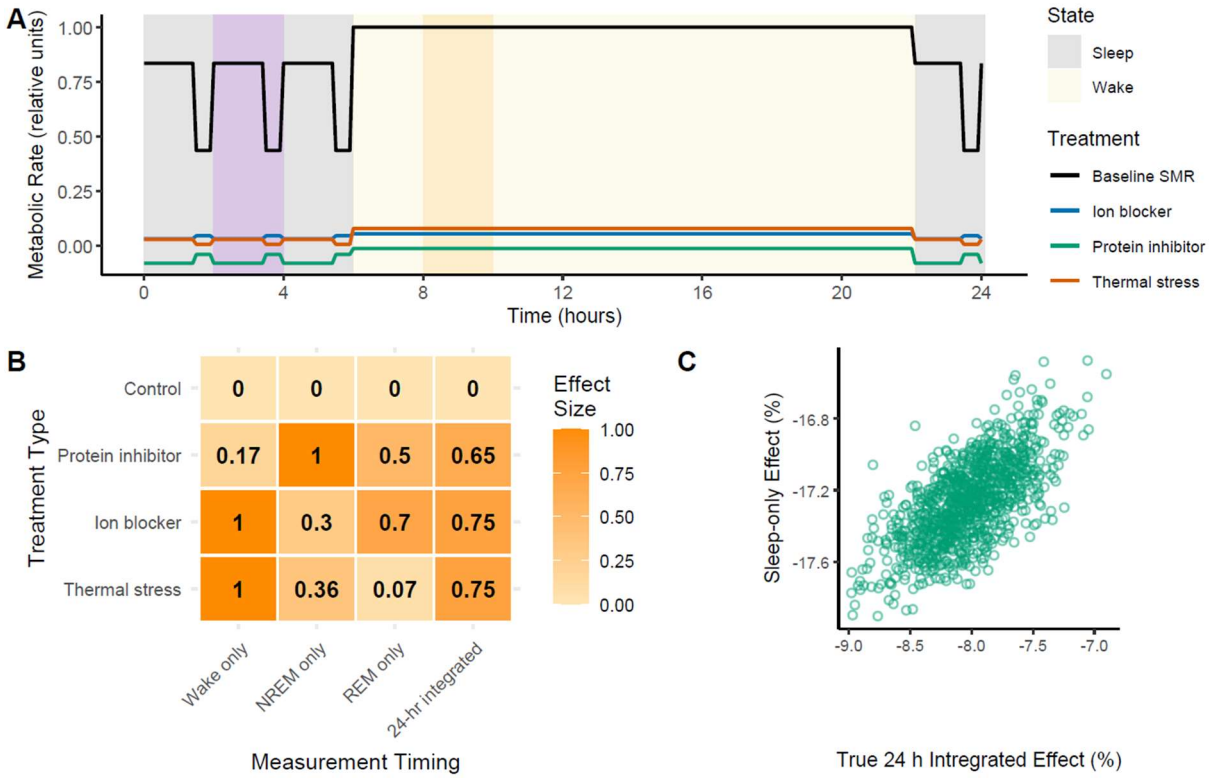

Figure 5. Consequences of state-dependent maintenance costs for detecting treatment or environmental effects on metabolic rate. (A) Simulated 24-hour timeline of standard metabolic rate (SMR) under baseline and treatment conditions. Black line represents the shifting SMR baseline, calculated from the integrated costs of all maintenance processes, which vary with behavioural state (wake, NREM sleep, REM sleep; Figure 1). Coloured lines show treatment-induced metabolic costs for three hypothetical experimental manipulations: a protein synthesis inhibitor (green), an ion transport blocker (blue), and thermal stress (orange). Costs for each process are relative to baseline SMR at any timepoint, and relative to the background cost of each process (0 = no change from process background). Shading denotes periods of wakefulness (light yellow) and sleep (grey); purple represents a period of sleep metabolic rate measurement; dark yellow represents a period of wake measurement. (B) Heat map showing how treatment effects depend on the timing of SMR measurements. Values indicate the proportion of maximum treatment effect that would be detected if sampling were restricted to wake, NREM, REM, or a 24-hour integrated period. Each treatment shows maximum effect size (1.0) during the sleep-wake state when the targeted maintenance process is most active: protein synthesis during NREM sleep, ion regulation during wake, and thermoregulation during wake. (C) Results of simulation showing the discrepancy between estimated treatment effects of a protein-synthesis inhibitor, based on sleep-only sampling and the true 24-hour integrated effect, across 200 individuals over five days each (Supplement 3). Each point represents a single individual-day. Treatment effects of the hypothetical protein inhibitor were applied only to sleep-active processes, and individual variation in sleep duration and REM:NREM ratio causes systematic bias when sampling is restricted to sleep, relative to the total time-integrated SMR. Both axes show treatment effects as percent change relative to the total time-integrated value for SMR.

Ecological and Evolutionary Consequences

Sleep-Wake Partitioning as an Evolutionary Constraint

The partitioning of maintenance functions across sleep-wake states has implications for evolutionary constraints on maintenance metabolism. If specific maintenance processes are prioritized during specific sleep stages, then their expression is limited by the amount and architecture of sleep that an organism can accommodate. For example, enhanced cellular repair or immune function may require increased NREM sleep, but this may be evolutionarily constrained in species facing high predation risk or strong selection for vigilance [31]. Conversely, species with consolidated or prolonged sleep may afford investment in metabolically costly processes that occur during sleep, facilitating different metabolic adaptations. Overall, selection on maintenance efficiency could favour specific sleep patterns, while ecological sleep constraints may limit evolutionary options for maintenance costs. These linkages could generate relationships among sleep architecture, behaviour, and physiology that may remain undetectable if maintenance costs are measured without accounting for sleep-wake state, masking potential roles for sleep architecture as a hidden axis of life-history trade-offs and physiological trait evolution.

Repeatability and Heritability of Metabolic Traits

Repeatability describes trait consistency within individuals, while heritability estimates the proportion of trait variation that is due to genetic differences. Both measures determine whether traits like SMR are likely to respond to selection. However, if metabolic measurements are influenced by unmeasured variation in sleep architecture, SMR estimates may reflect transient sleep-wake states, as opposed to stable physiological traits. This would artificially inflate within-individual variability and reduce repeatability, especially if sleep patterns fluctuate across measurement days. Conversely, if sleep architecture is stable within individuals but varies consistently among individuals, the corresponding sleep-biased SMR estimates may appear more repeatable than the true 24-h integrated maintenance expenditure[32,33] and SMR heritability estimates may partially reflect genetic variation in sleep architecture rather than maintenance metabolism.

The Scaling of Metabolic Rates with Body Mass

For more than a century, researchers have sought general "scaling laws" describing how metabolic rate changes with body size[34–37]. However, accumulating evidence suggests that metabolic scaling exponents vary systematically with species lifestyle[38], thermal environment[39,40], ontogenetic stage[41], and activity level[38,42]. Our framework suggests that part of this variation may stem from overlooked differences in sleep-wake architecture across body sizes. Traditional scaling models assume SMR reflects consistent maintenance processes across organisms, but if these processes are differentially expressed across sleep-wake states, and sleep-state partitioning varies with body size, SMR scaling may include hidden biases. Smaller animals sleep more but have shorter, fragmented cycles and higher relative SMRs[13], meaning that over a given time interval, their SMR measurements may sample a broader range of slee-wake states. Larger animals tend to exhibit reduced but more consolidated sleep[13], potentially producing more state-specific but less representative SMR measurements. Furthermore, if specific maintenance functions scale differently with body mass and are differentially regulated across sleep states, apparent scaling exponents may reflect sampling window bias or process-specific measurement bias, rather than fundamental physiological rules, introducing unrecognized variability in metabolic scaling.

Ontogenetic Patterns and Developmental Energetics

Sleep architecture changes markedly during early development[43]. Altricial mammals can spend 80-100% of their early postnatal sleep in REM, whereas precocial species maintain relatively stable, adult-like sleep-state proportions from birth or hatching[44]. Because REM sleep is associated with particularly low metabolic activity, SMR measured during sleep in young altricial animals may underestimate true maintenance costs, potentially confounding species comparisons of developmental energetics or intraspecific metabolic scaling. Moreover, because costly processes such as protein synthesis and neural plasticity are preferentially active during sleep,

SMR estimates incorporating wake periods may underestimate the energetic costs of growth and brain development in young animals. While sleep architecture continues to change across entire lifespans, exact patterns differ among species. In humans, for example, sleep quality and consolidation decreases with aging, while laboratory rodent studies show increasing sleep duration and intensity with age[45,46]. Overall, age-related changes in sleep may create measurement biases that vary both across species and throughout lifespans, with potential implications for comparative studies of aging and senescence.

Links Between Metabolic Traits and Behaviour

Over the last two decades, interest has surged in quantifying relationships between SMR and behaviour[47,48]. However, if maintenance costs differ systematically between sleep and wakefulness, then measuring SMR during sleep and behavioural data during wakefulness is effectively sampling from different physiological baselines. As such, using SMR values from one state to predict behaviour may be meaningless without cross-state correlation in total maintenance costs. This issue may also obscure observations of behavioural syndromes. Differences in boldness, vigilance, or exploration could influence how individuals sleep within respirometry setups, with more timid individuals sleeping less deeply or more briefly. These differences would affect measured SMR, creating spurious correlations between metabolism and wake-measured behavioural phenotypes driven by sleep-wake state variation during SMR measurement. In addition, SMR-behaviour correlations commonly vary across environmental gradients like temperature or food availability[49], and this phenomenon is typically interpreted as an environmentally induced shift in trait covariance. However, some observed covariance shifts may reflect artefacts from inconsistent sleep state partitioning during SMR estimation, especially if environmental factors alter sleep architecture. Additionally, single-state SMR measurements only capture a subset of specific maintenance processes, so correlation variations may be due to shifts among sleep-predominant maintenance processes, while relationships involving waking processes may remain unobserved and therefore undetectable.

Recent work also shows that social stress and dominance hierarchies can alter individual sleep architecture, with dominant and subordinate individuals differing in REM duration and sleep fragmentation[50]. Aside from direct effects of sleep variation on metabolic costs and recovery from conflict, the widely observed associations between SMR and aggression or dominance[51] may be partly mediated by variable sleep-wake states during SMR measurement between dominants and subordinates, and not solely due to intrinsic metabolic phenotypes.

Thermal Performance Curves of Aerobic Scope

Thermal performance curves for aerobic scope are widely used to assess physiological limits of ectotherms under different thermal environments, identify thermal optima, and predict climate change vulnerability[52]. However, sleep-wake partitioning of SMR could introduce unacknowledged error in aerobic scope estimation across temperatures. Since SMR is often measured during resting periods that may include varying sleep proportions, temperature-driven shifts in sleep duration or architecture may systematically bias SMR estimates. Additionally, if animals sleep more deeply or longer at certain temperatures, and if these sleep states involve lower metabolic costs, SMR measured during those periods will be artificially low. This would inflate aerobic scope estimates due to reduced maintenance costs captured during sleep-heavy measurement windows, as opposed to true physiological optimization. Such biases could affect thermal performance curves by exaggerating peaks, shifting optima, or confounding performance limits, all due to effects of temperature on sleep architecture in addition to direct effects on SMR itself. Moreover, interspecific or interindividual comparisons could become complex if temperature sensitivity of sleep differs among taxa or individuals, as these differences could appear as variation in aerobic performance instead of measurement artefacts.

Calming Effects of Conspecifics and Social Buffering of Stress

Numerous studies report that the presence of conspecifics reduces measured metabolic rates in social species, often interpreted as a calming or stress-buffering effect[53,54]. However, if these measurements are taken during quiescent periods (e.g. night time), an alternative explanation is that conspecific presence modulates sleep architecture[55], leading to changes in the proportions of REM and NREM sleep being observed. For example,

decreased risk perception in the presence of conspecifics may lead to longer or deeper NREM sleep[56], or less fragmented sleep cycles, thereby reducing the contribution of metabolically costly waking states during the SMR measurement window. Conversely, isolation or social stress might fragment sleep or increase the time spent awake, elevating apparent SMR. This would mean that the observed metabolic changes may reflect indirect shifts in the sleep-state composition during measurement instead of direct decreases in maintenance or routine costs via stress reduction. If true, this reinterpretation could alter how we view social buffering effects and their implications for energy budgets in group-living species.

Improving Our Understanding of Environmental Change

Our framework suggests environmental change research faces two challenges: (1) overlooking real physiological effects on specific maintenance processes; and (2) misinterpreting sleep-wake changes as metabolic impacts. Environmental stressors may cause metabolic effects confined to specific sleep-wake states, due to effects on specific maintenance processes, but researchers could miss these impacts if measuring metabolic rates during the wrong period. For example, aquatic acidification or salinity changes can alter ion regulation in marine organisms[57], but since this may primarily occur during wakefulness, sleep-only measurements could underestimate associated energetic costs and impacts. Conversely, some reported environmental effects on metabolism may actually reflect sleep architecture changes rather than direct physiological costs. Noise pollution, light pollution, or habitat disturbance can fragment sleep or alter time spent in different sleep states during measurement periods[58], leading to apparent "metabolic effects" that represent shifts in sampled sleep-wake states instead of true changes in underlying maintenance costs. Climate warming might simultaneously impose real thermoregulatory costs (primarily during wakefulness) while altering sleep duration or quality, confounding direct temperature effects with sleep-mediated measurement window changes. Indeed, environmental factors may operate through multiple pathways: direct effects on maintenance processes, indirect effects through sleep-wake architecture changes, and measurement artifacts from state-dependent sampling (Figure 1). Disentangling these mechanisms will be important for understanding true physiological impacts of environmental change.

Re-Evaluating Basal Metabolism: A Path Forward

Given the potential for substantial error in SMR estimation due to unaccounted variation in sleep-wake architecture, it is critical to develop strategies that can mitigate or quantify this source of bias. Here we outline a range of possible approaches, from the ideal but logistically demanding to more feasible alternatives. The most appropriate option will also depend on factors such as cost and alignment with specific research goals.

Defining an Integrated Daily Maintenance Expenditure

Although logistically untenable in most situations, at least for now, a “gold standard” for estimating maintenance metabolism would move beyond the assumption of a static maintenance cost and to capture an integrated daily maintenance expenditure (IDME): the total energetic cost of maintenance processes across all sleep-wake states over a full circadian cycle. This would ideally involve continuous or high-resolution measurement of metabolic rate across 24 hours (or longer), with concurrent classification of sleep-wake state to allow state-specific partitioning of energy use. Depending on the organism, this could be achieved using respirometry or doubly labeled water paired with electrophysiological, behavioural, or indirect indicators of sleep-wake state[59] (e.g. EEG in mammals or birds, accelerometry or infrared video tracking in fishes or invertebrates). The goal would be not just to average metabolic rate across time, but to weight it by the proportion of time spent in each state and the specific processes active during those periods. To be clear, this is logistically challenging, or even impossible, with existing technology and especially in non-model organisms. However, such an approach would offer the most ecologically and evolutionarily relevant estimates of baseline metabolism, reflecting how organisms actually allocate energy to maintenance functions over time, rather than how they perform in an artificially static physiological state.

A conceptual model for estimating integrated daily maintenance expenditure (IDME) can be formalized as a time-weighted sum of state-specific metabolic rates:

$$\mathrm{IDME} = \sum_{i=1}^{n} \mathrm{M}i \cdot \mathrm{T}i$$

M*i* = mean metabolic rate during state *i* (e.g., wakefulness, NREM sleep, REM sleep)

T*i* = proportion of the 24-hour period spent in state *i* (such that ∑Ti=1)

n = number of behavioural states considered (typically three for mammals and birds: wake, NREM, REM)

This equation assumes that each behavioural state has a characteristic metabolic rate and that total maintenance cost is the sum of these rates scaled by the time spent in each state. If empirical data are available, M*i* can be measured directly; otherwise, state-specific correction factors can be applied to standard SMR values. For example, if quiet wake SMR is used as a baseline, literature-derived multipliers (e.g. 0.83 for NREM, 0.44 for REM, Table S1) can be applied to approximate taxa and state-specific contributions. This substitution is not ideal, but is conceptually analogous to how generalised metabolic scaling exponents are often applied to datasets to correct for the effects of body mass, when data for that exact species or size range is not available. Similarly, the time allocation terms (T*i*) can be derived from electrophysiological data (e.g., EEG/EMG recordings in mammals), automated behavioural tracking (e.g. posture analysis or motion sensors), or estimated from published sleep architecture profiles for a given species. In cases where species-specific data are unavailable, approximate values can be obtained from related taxa or scaled using known allometric or ecological correlates of sleep duration. This formulation allows estimation of daily maintenance costs in a way that reflects both temporal partitioning of behaviour and differential expression of maintenance functions across states. However, if generalised estimates for sleep-state multipliers are being used, this would not address biases in SMR estimation that occur at the individual level, due to among-individual variation in sleep architecture [32,33]

Enhancing Current Methods Through Sleep-State Inference

Existing approaches may be improved by developing better inferences about the sleep-wake state during SMR measurement. These refinements may serve as intermediate solutions that improve the biological realism of SMR estimates, particularly in systems where direct state identification is challenging but behavioural and metabolic data are available at high temporal resolution. In this way, existing methodologies can evolve toward more informed estimates of maintenance metabolism, even in the absence of full IDME capability.

For instance, measuring oxygen uptake across full circadian cycles may help capture a broader range of sleep-wake states[60], although without clear identification of which states are being recorded, estimates will remain biased toward lower-cost sleep phases. This is particularly relevant in intermittent-flow respirometry, where the method of SMR calculation itself may introduce hidden state-associated bias. Approaches such as using a lower quantile of MO_2 values to define SMR[61] may disproportionately represent sleeping periods, particularly in individuals with greater sleep needs, leading to underestimates of true time-integrated SMR. Similarly, the use of the mean of the lowest normal distribution[61] will not resolve this issue unless data span multiple circadian cycles; even then, the resulting SMR estimate is likely to reflect metabolically quiescent phases such as REM sleep.

However, these same methods could be refined to disentangle sleep- and wake-dominant energy costs. If repeated patterns emerge across the diel cycle – such as distinct frequency distribution peaks in oxygen uptake values[60], these may correspond to specific sleep-wake states and could be used to partition SMR into state-specific components. Pairing such analyses with infrared video tracking or automated motion detection would allow coarse classification of behavioural state, helping to align metabolic estimates with sleep-wake architecture. In aquatic systems using intermittent-flow respirometry [62,63], another promising strategy would be

to pair activity measurements with oxygen uptake slopes on a per-phase basis, generating a large number of slope-activity pairs from which could be used to calibrate the relationship between spontaneous movement and oxygen uptake. This would allow researchers to extrapolate to an estimated SMR at zero activity, yielding a more realistic estimate of maintenance costs during wakefulness. However, this approach requires the ability to quickly quantify and align activity with each oxygen uptake measurement. As such, it highlights the need for improved video acquisition systems and automated analytical pipelines capable of extracting activity metrics at high temporal resolutions.

State-Specific Metabolic Profiling

While IDME offers the most comprehensive estimate of baseline energy use, it is not always necessary, or even desirable, depending on the research question. In many cases, state-specific SMR measurements may be the most appropriate approach, particularly when the behavioural or physiological state during measurement aligns with the focal process under investigation. For example, if the aim is to understand energy constraints on locomotion, predator avoidance, or other active behaviours, SMR and aerobic scope measured during quiet wakefulness may offer more meaningful insight than a time-averaged value diluted by metabolically depressed sleep phases. Conversely, studies focused on immunity, cellular repair, or protein synthesis may benefit from sleep-specific SMR measurements, particularly if these processes are known to be upregulated during NREM sleep[12]. Instead of prescribing a single ideal measurement strategy, we suggest that researchers explicitly match their SMR measurement window to the behavioural or ecological state most relevant to their hypothesis, and interpret their results accordingly. This state-matching approach offers a pragmatic and conceptually sound alternative when full 24-hour measurement is not feasible.

Understanding and Acknowledging the Extent of Bias

In some cases, simply acknowledging and quantifying these types of bias may be sufficient. Or, if researchers can confirm (or reasonably assume) that maintenance costs are similar between the measurement window and the behavioural context of interest (e.g. day vs. night), then some level of state-related error may be tolerable. After all, respirometry already involves accepted approximations – such as using oxygen uptake as a proxy for true energy expenditure[62]. Our framework highlights an additional, but to date overlooked, source of variation that can now be assessed and, where necessary, addressed.

Avenues for Future Research

Confirming the extent to which SMR fluctuates with sleep-wake state across individuals, species, and contexts will require targeted empirical research (Table 1). For example, many of our estimates of state-partitioning are indirect and based on up or down-regulation in organ or tissue functioning, as opposed to direct measures of state- and process-dependent energy expenditure[1,12]. Increased direct measurements of metabolic rate across sleep-wake states, especially using high-resolution methods that can distinguish NREM and REM, are needed to confirm the predicted shifts in energetic allocation. In addition, naturally divergent sleep architectures across species or ecotypes offer opportunities to test whether state partitioning contributes to apparent interspecific differences in SMR. For example, comparing high-REM and low-REM phenotypes, or animals exposed to chronically fragmented vs. consolidated sleep[31,56], may help disentangle true physiological divergence from measurement artefacts. Contrasting diurnal vs. nocturnal mammals, cave vs surface-dwelling morphs of the same species (e.g. cavefish), or animals exposed to varying environmental conditions that alter sleep architecture[14,15] could also offer powerful systems for testing whether variation in REM/NREM balance corresponds to predictable shifts in measured metabolic rate.

Further, little is currently known about how transitions between sleep states affect maintenance costs. Our models implicitly assume a rapid or instantaneous switch in physiological function when transitioning between sleep states, but this is also unlikely to reflect biological reality. Transitional periods may involve partial or overlapping activation of maintenance processes, and in species with highly fragmented sleep, carry-over effects between states could meaningfully alter the relative costs and timing of metabolic costs of specific processes[12,14].

Understanding these transitional dynamics will be essential for refining both empirical measurements and modelling approaches.

Conclusions

While SMR is often treated as a within-individual physiological constant, we suggest that this assumption is rarely met in reality. Across taxa, maintenance processes are partitioned across sleep and wakefulness, and even within individuals, sleep architecture can change with size, context, and environment. These effects introduce a fundamental source of physiological variation that is overlooked in metabolic studies, but could systematically bias the estimation, interpretation, and application of SMR across fields.

It is worth asking: *under what conditions would sleep-state partitioning of maintenance costs not affect the measurement or interpretation of SMR?* For this to be the case, several biologically implausible criteria would need to be met. First, all maintenance processes would need to operate at equivalent intensity across wakefulness, NREM, and REM sleep, or at least have their sum total of energetic costs be equal across these states. As we have discussed, this condition is at odds with well-documented down-regulation of some maintenance processes during sleep and upregulation of others. Second, individuals would need to exhibit minimal among- and within-individual variation in daily sleep-wake cycles and sleep architecture (e.g., REM:NREM ratios), such that any fixed measurement window captures the same metabolic profile across animals and days. Finally, downstream uses of SMR estimates – such as comparisons across individuals or species, or calculations of aerobic scope or energy budgets – would need to be unaffected by any sleep-wake biases in SMR measurements and involve only the same behavioural or physiological states in which SMR was measured.

Taken together, these conditions are not only unlikely, but biologically unrealistic. In light of growing evidence for state-dependent variation in maintenance metabolism, it is no longer tenable to assume that SMR reflects a fixed energetic baseline. Moving forward, researchers should consider if and how sleep-wake state is accounted for in metabolic measurements, and the implications of state-limited sampling on the traits, comparisons, and inferences they seek to draw. By abandoning the fiction of a constant metabolic baseline, we can build a more accurate and biologically grounded understanding of organismal energetics.

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685 **Table 1:** Empirically testable predictions arising from a state-partitioned view of standard metabolic rate
686 (SMR). Implications range from experimental design to broader evolutionary and ecological theory.

#	Prediction	Rationale	Possible Test	Implications
1	SMR measured during wakefulness (e.g. extrapolating MR to zero activity) will exceed SMR measured during sleep.	Wakefulness involves higher costs for thermoregulation, sensory processing, and ion gradients.	Compare SMR from sleep vs. quiet wake in same individuals.	Highlights importance of behavioural state control in metabolic protocols, attempts to correlate MR with behaviour.
2	Endotherms will show larger errors in SMR estimation during sleep than ectotherms.	Thermoregulatory effort is downregulated during sleep in endotherms.	Compare state-specific SMR across endo- and ectotherms.	SMR bias may differ systematically across taxa, complicating cross-species comparisons.
3	Species or individuals with higher REM sleep proportions will show greater underestimation of SMR when measured during sleep.	REM is metabolically less costly; higher REM skews measured values downward.	Measure REM proportion and compare to error magnitude.	REM duration may act as a hidden source of inter-individual or interspecies variation.
4	Individuals or species with larger or more neuron-dense brains will show a greater error in SMR estimation during sleep.	More brain ion regulation as a maintenance cost during wake.	Compare state-specific SMR across species with different brain sizes or neuron densities.	SMR variation within and across species may be partially due to biases associated with misestimation of total brain costs.
5	Trait correlations with SMR (e.g. boldness, activity) depend on sleep architecture during measurement.	Sleep traits modulate the underlying maintenance processes being measured.	Control for or stratify analyses by sleep profile.	Some reported physiological-behavioural links may be artefacts of sleep-state variation.
6	Apparent context-dependent shifts in SMR-trait relationships may reflect altered sleep architecture, not true metabolic plasticity.	Environmental variables (e.g. temperature) affect sleep and thereby SMR estimates.	Simultaneously track sleep and SMR across environments.	Reframes some plasticity findings as measurement artefacts, not physiological change.

7	Species with high maintenance demands (e.g., immune or neural activity) will exhibit longer or more consolidated sleep.	Sleep permits efficient expression of these functions.	Correlate maintenance traits and sleep duration across species.	Suggests evolutionary linkage between sleep architecture and physiological capacity.
8	Sleep architecture and related SMR estimation error will show a phylogenetic signal.	Sleep-metabolism integration may follow evolutionary trajectories.	Map traits and error onto phylogenies.	Affects how metabolic traits are interpreted in a comparative or macroevolutionary context.
9	Environmental factors that fragment sleep will increase measured SMR.	Wake periods during measurement inflate apparent baseline metabolism.	Compare SMR and sleep in disturbed vs. controlled settings.	Redefines “stress effects” on metabolism as partly sleep-modulated.
10	Treatment effects on metabolism will be state-dependent, with sleep-active interventions showing stronger effects during sleep measurements and wake-active interventions during wake measurements.	Maintenance processes are temporally partitioned; interventions affecting specific processes are only detectable when those processes are most active.	Compare SMR responses to stress during sleep vs. wake measurements.	Inconsistent treatment effects may reflect measurement timing instead of biological variation.

Supplement 1. State-Dependent Partitioning of Maintenance Energy Costs Contributing to SMR

Table S1. Estimated state-dependent partitioning of energy required for maintenance processes and the putative contribution of each process to basal/standard metabolic rate (SMR). Depending on the process, state-partition values were derived from a combination of direct physiological measurements and indirect evidence from gene expression, metabolic tracer studies, and neuroimaging. In most cases, contributions to SMR were estimated from published estimates, but these are typically based on data collected during a single state (e.g., sleep or quiet wakefulness); as a result, they may not reflect the full energetic cost of a function across the full sleep-wake cycle. Uncertainty rankings indicate the strength of empirical support (Low = well-quantified; Moderate = indirect inference; High = speculative). We follow Rolfe & Brown (1997)[1] in recognizing that organ-level 'service functions' (e.g., liver detoxification, heart functioning, motor control of breathing) contribute significantly to whole-body energy use. However, the energetic costs of these functions are already at least partially represented within the cellular processes included in our table (e.g., ion regulation, protein synthesis, substrate cycling), and their state-dependent partitioning is difficult to resolve. For this reason, we do not treat service functions as a separate category.

	Partitioning (%)			Total SMR	State SMR Contribution (%)				
Maintenance Process	Wake	NREM	REM	Contribution (%)	Wake SMR	NREM SMR	REM SMR	State Uncertainty	SMR Uncertainty
Ion Gradient (Brain)	50	15	35	15	7.5	2.25	5.25	Moderate	Low
Ion Gradient (Peripheral)	60	35	5	12	7.2	4.2	0.6	Moderate	Moderate
Protein Synthesis	10	60	30	18	1.8	10.8	5.4	Low	Low
Immune Surveillance	25	60	15	7	1.75	4.2	1.05	Moderate	High
Thermoregulation	70	25	5	15	10.5	3.75	0.75	Low	Moderate
Neural Plasticity	20	45	35	4	0.8	1.8	1.4	Low	Moderate
Sensory Processing	90	5	5	5	4.5	0.25	0.25	Low	Moderate
Glymphatic Clearance	30	60	10	5	1.5	3	0.5	Low	High
Nitrogenous Waste	45	35	20	2	0.9	0.7	0.4	Moderate	Low
Substrate Cycling	60	20	20	7	4.2	1.4	1.4	Moderate	Low
Gluconeogenesis	35	60	5	4	1.4	2.4	0.2	High	Low
Residual Background	33	33	33	6	1.98	1.98	1.98	Moderate	Moderate
TOTAL				**100**	**44.03**	**36.73**	**19.18**		

Neural Plasticity / Memory Consolidation

Neural plasticity refers to the restructuring of synaptic connections through strengthening, weakening, or remodeling, and involves energy-intensive activities such as protein synthesis, receptor modulation, and neural reorganization. Based on *in vivo* ATP imaging during NREM, it is estimated that ~10% of cortical ATP during sleep is directed toward plasticity-related processes[2]. When scaled to the cortex's overall contribution to total SMR (~20% in humans; [1]) and expanded to account for additional plasticity demands in other brain structures, we estimate that neural plasticity contributes approximately 4% of whole-body SMR. Plasticity is not distributed evenly across behavioural states; as described by the synaptic homeostasis hypothesis[3], synaptic strength accumulates during wakefulness as new information is encoded, but is selectively downscaled during NREM sleep to restore efficiency. These NREM-linked changes are supported by dendritic calcium bursts that coincide with sleep spindles and signal localized increases in energy use[4]. REM sleep contributes a second wave of plasticity, characterized by hippocampal-cortical replay of activity patterns from prior waking experience, thought to underlie memory consolidation[5]. Reflecting the combined but distinct contributions of NREM and REM, we allocate 45% of plasticity-related energy use to NREM, 35% to REM, and 20% to wake.

Sensory Processing

Sensory processing is a metabolically active function of the cortex, particularly during wakefulness when animals must monitor and respond to external stimuli. Sensory cortical regions (e.g., visual, auditory, and somatosensory cortices) represent a major share of cortical volume and synaptic activity during wakefulness. Given that the cerebral cortex accounts for ~20% of whole-body SMR[1], and that a significant portion of cortical signaling is dedicated to sensory integration during wake, it is reasonable to estimate that sensory processing contributes

~3-5% of SMR. This does not include energy used by subcortical sensory relays (e.g. thalamus) or alertness-related sensory gating. To conservatively account for these components and reflect continuous sensory engagement during wake, we assign 5% of SMR to sensory processing. Sensory cortical activity declines significantly during NREM sleep[6] and is largely disengaged during REM sleep despite high overall brain activity[7].

Immune Surveillance and Modulation

Estimates of the energy cost of baseline immune function are not available, but both theoretical and empirical data suggest that constitutive immune processes (e.g., leukocyte maintenance, low-level cytokine signaling, and general immune readiness and surveillance) represent a non-trivial portion of the resting metabolic rate[8,9]. Although most empirical work focuses on activated immune responses (which can raise metabolism by 15-30%), baseline maintenance of immune competency likely involves continuous low-level metabolic investment from lymphoid organs and leucocyte activity, suggesting these costs are present even in healthy individuals[10]. Based on this, a conservative estimate of ~7-8% of SMR for baseline immune metabolism is biologically plausible, though uncertainty is high due to the lack of direct quantification. Evidence suggests that baseline immunity is not uniformly distributed across the sleep-wake cycle[11,12]. Indeed, circadian activity of some immune components (e.g. cytokines) appears to have neuromodulatory roles that regulate sleep, in addition to their direct immunological function[13]. Studies in humans and other animals also show that early NREM sleep coincides with a hormonal response that favours immune expression, characterized by low cortisol and high growth hormone, with NREM sleep supporting adaptive immune functions such as antigen presentation, leukocyte activity, and T-cell activity[14]. Disruptions to sleep reliably alter immune gene expression[10], further suggesting that sleep facilitates important immune processes. In contrast, REM is thought to contribute little to baseline immune functioning, as it coincides with rising cortisol, reduced growth hormone, and increased sympathetic activation[14]. Together, these findings suggest NREM sleep is the primary period of baseline immunological maintenance and coordination, while wake supports more peripheral immune readiness and REM contributes minimally (Table 1). However, the precise energetic costs of these processes remain uncertain and likely vary across species, tissues, and immune functions.

Glymphatic Metabolite Clearance

Glymphatic clearance is the convective exchange of cerebrospinal and interstitial fluid that facilitates metabolic waste removal from the brain and is upregulated during sleep. While the energetic cost of this process has not been directly quantified, it likely imposes appreciable ATP usage associated with glial activity, cerebrospinal fluid movement, and vascular-neural coupling. Based on this rationale, we tentatively estimate glymphatic function to contribute approximately 5% of standard metabolic rate (SMR), reflecting the likely contribution of glial and vascular processes during peak glymphatic activity, but should be interpreted cautiously due to the absence of direct measurements. Glymphatic function is known to be strongly state-dependent. During NREM sleep, there is a 2-fold increase in clearance compared to wake[15], then a reduction during REM[16]. Based on this evidence, we assign 60% of glymphatic metabolic activity to NREM sleep, 30% to wake, and 10% to REM.

Nitrogenous Waste Processing

It has been estimated that nitrogenous waste management contributes approximately 2% of standard metabolic rate in mammals[1]. The temporal expression of nitrogenous waste processing exhibits pronounced circadian partitioning linked to both protein turnover cycles and kidney function rhythms, though the evidence of changes in metabolic costs remain mostly indirect and inferred through changes in kidney activity. Glomerular filtration rates, for example, display strong circadian rhythmicity, with maximum values during daytime and minimum values at night[17]. Experimental evidence demonstrates that renal hormonal control differs fundamentally between sleep and wake states[18]. During sleep, aldosterone pulses are mainly related to plasma renin activity (PRA) oscillations, whereas during waking periods, aldosterone pulses are primarily associated with cortisol

pulses. Furthermore, PRA shows oscillations strongly linked to REM-NREM cycles, with NREM sleep linked to increasing PRA and REM sleep associated with decreased PRA[18]. These state-dependent differences in renal regulation suggest that metabolic costs of nitrogenous waste processing vary across states. Based on these considerations and documented circadian variations in hepatic and renal function, we provisionally assign 45% of nitrogenous waste processing costs to wakefulness, 35% to NREM sleep, and 20% to REM sleep, though uncertainty surrounds these estimates given limited direct quantification of state-dependent waste metabolism across different taxa.

Substrate Cycling

Substrate cycling refers to ATP-consuming biochemical loops involving opposing metabolic pathways (e.g., lipolysis and lipogenesis, triglyceride and fatty acid turnover) that allow rapid shifts in fuel usage and metabolic regulation. Rolfe & Brown (1997) estimated that substrate cycling contributes approximately 7.5% of SMR, based on modelling cycles across liver, muscle, and adipose tissue. More recent studies (e.g.[19]) have shown that substrate switching – indicated by fluctuations in respiratory quotient – continues across the sleep-wake cycle, particularly during transitions in and out of REM sleep, supporting the view that although substrate cycling is most pronounced during waking periods, when fuel demands are highest, it is also modulated by sleep. NREM sleep is associated with increased lipolysis, growth hormone release, and free fatty acid availability, all of which support ongoing hepatic and adipose substrate cycling[20,21]. During REM sleep, bursts of sympathetic activity and increased brain glucose uptake further sustain metabolic flexibility. Based on this, we assign 60% of substrate cycling energy use to wakefulness, and 20% each to NREM and REM, reflecting both continuous background cycling.

Gluconeogenesis

Gluconeogenesis and glycogen metabolism are essential components of energy homeostasis, particularly during fasting or extended periods without food intake, such as overnight sleep. Although their energetic cost is often overlooked, Rolfe & Brown (1997) estimated that gluconeogenesis could account for 3-6% of SMR in mammals. This includes both hepatic glucose production and brain glycogen turnover, which is known to fluctuate across sleep-wake states. According to the glycogenetic hypothesis, brain glycogen is depleted during wakefulness due to high neuromodulatory activity and replenished during NREM sleep, a process supported by increased glycogen synthase activity and reduced sympathetic tone[22]. In contrast, glycogen breakdown and gluconeogenic demand rise during wakefulness, particularly during prolonged wake or energy stress, when glucose needs increase in both peripheral tissues and the brain. REM sleep appears to contribute minimally to net glycogen turnover, although bursts of neuronal activity may elevate local glucose oxidation. Based on these patterns, we estimate the contribution of gluconeogenesis and glycogen metabolism to SMR at 4%, and partition the cost as 60% to NREM, 35% to wakefulness, and 5% to REM, reflecting the anabolic and catabolic phases of carbohydrate cycling across the sleep-wake cycle.

Residual Background Processes

A portion of standard metabolic rate reflects baseline cellular functions that are essential for survival but not strongly influenced by behavioural state. Rolfe and Brown (1997) estimated that these background processes account for approximately 5-8% of SMR, depending on the species and tissue type. This component is thought to remain relatively constant across sleep-wake states, as it reflects the irreducible energetic cost of maintaining basic membrane potential, resting mitochondrial function, and low-level enzymatic activity. For this reason, we assign a value of 6% of SMR to background maintenance functions and apply it evenly across wake, NREM, and REM.

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Supplement 2: Simulations of State-Dependent SMR and Experimental Error

Estimating Error in SMR from State-Limited Sampling

To quantify the potential error introduced when basal metabolic rate (SMR) is measured in only a single behavioural state (e.g., sleep or wakefulness), we produced a deterministic model using R (v4.4.0) to simulates how state-dependent partitioning of maintenance processes can affect SMR estimates.

We first defined 12 core physiological maintenance processes contributing to SMR (e.g. ion gradient maintenance, thermoregulation, protein synthesis), based on values derived from literature estimates and physiological reasoning where required (see Table S1). Each process was assigned: (1) an estimated contribution to total SMR (%); and (2) proportional activity levels across wake, non-REM (NREM) sleep, and REM sleep. These activity levels reflect known or inferred patterns of state-dependent up- or down-regulation (e.g. thermoregulation is active primarily during wake; protein synthesis peaks during NREM sleep).

We then simulated two contrasting SMR estimation approaches: one where measurements are taken exclusively during wakefulness ("wake-only"), and another where measurements are taken exclusively during sleep ("sleep-only"). In each model, metabolic costs are further partitioned between NREM and REM sleep, varying according to the proportion of total sleep time the animal spends (or would normally spend, in the case of the “wake-only” panel) spent in REM (ranging from 10% to 50%). The true SMR for a given individual or species was modelled as the weighted sum of state-specific SMR values over a full 24-hour period, representing their proportion of time spent awake versus asleep. Percent error was calculated as the absolute deviation of the wake-only or sleep-only estimate from the true time-integrated SMR.

Simulating the Effects of State-Dependent Partitioning on SMR Estimation

To explore how neglecting sleep-wake partitioning may bias estimates of SMR and aerobic scope, we constructed a stochastic individual-based simulation in R (v.4.4.0). The model generated a simulated population of 200 individuals, each undergoing five repeated measurements across five separate days (totaling 1000 observations). The true 24-h integrated SMR for each individual was drawn from a normal distribution centered at 0.35 (arbitrary units) with a standard deviation of 0.05, producing approximately 2.5-fold variation across the population.

To introduce biologically plausible within-individual consistency in sleep patterns, each individual was assigned a baseline trait value for total sleep duration and REM sleep proportion. These values were drawn from normal distributions (mean = 10 h, SD = 1 for total sleep; mean = 0.25, SD = 0.05 for REM proportion, constrained to 0.05-0.5). On each of the five simulated nights, an individual's sleep architecture was modeled by generating nightly values from normal distributions centered around their baseline, with additional night-to-night stochasticity. Specifically, daily total sleep duration was drawn from a normal distribution centered on the individual's baseline, with a smaller standard deviation (e.g. SD = 0.5 h), while daily REM proportion was similarly drawn from a normal distribution centered on the individual's REM baseline (SD = 0.025), constrained between 0.05 and 0.5. This structure preserved among-individual differences in sleep architecture while allowing plausible intra-individual variation across repeated measures.

Each SMR estimate during the 12-hour overnight measurement window was calculated based on the time-weighted expression of metabolic costs during REM and NREM sleep. These were assumed to reflect only partial contributions of the total maintenance processes expressed during waking hours, with multipliers derived from literature-based estimates, expressed as proportions of the maintenance costs while awake: 0.436 for REM sleep and 0.834 for NREM sleep (Table S1). These multipliers were applied to the proportion of time spent in each sleep stage during the 12-hour window, relative to the individual’s true SMR. Gaussian noise (SD = 0.01) was added to all measurements to reflect routine technical error during measurements.

A single value for maximum metabolic rate (MMR) was independently generated for each individual from a log-normal distribution with a mean centered around 5-fold the population mean SMR and modest variation (log SD = 0.01). For each individual on each measurement day, we calculated aerobic scope (AS) as the difference between MMR and multiple SMR estimates: the true time-integrated SMR (reflecting weighted contributions of wake, NREM, and REM states), the estimated SMR based on a simulated overnight sleep window, and three separate state-specific SMR values corresponding to wakefulness, NREM sleep, and REM sleep. This allowed us to compare how the SMR measurement state affects estimates of aerobic scope.

Estimating the impact of state-restricted SMR measurements on detection of treatment effects

To examine how the timing of SMR measurement influences estimates of treatment effects, we developed a simulation model based on the proportional contributions of various maintenance processes to total metabolic rate during wakefulness, NREM, and REM sleep. This model was designed to assess error occurring when experimental treatments differentially affect maintenance processes that are distributed across sleep-wake states, such that single-state measurements may fail to capture the full impact on maintenance energy use. By comparing the sleep-only estimates to the integrated 24-hour values, the model demonstrates how estimates of treatment effects vary not only with individual differences in sleep architecture but also as a function of the magnitude and state-specificity of the treatment effect. This provides a framework for understanding how common experimental constraints can lead to systematic underestimation or misrepresentation of the metabolic consequences of a treatment.

We simulated a protein inhibitor treatment that reduced the metabolic cost of protein synthesis by 50% in all three states. Baseline state-partitioned contributions for protein synthesis were 1.8% of BMR during wake, 10.8% during NREM, and 5.4% during REM (Table S1). These values were reduced by 50% in a modified dataset to represent a treatment that impairs protein synthesis across the full 24-hour period. All other maintenance processes remained unchanged between control and treatment datasets. The model assumes that protein synthesis is the only directly affected process and that its proportional contributions are state-dependent but additive.

To incorporate biological variability, we simulated 200 individuals, each measured on 5 separate days. On each simulated day, total sleep duration was drawn from a normal distribution with a mean of 8 hours per 24 h period, and a standard deviation of 0.5 hours. The proportion of sleep spent in REM was drawn from a normal distribution with a mean of 30% and standard deviation of 2.5%. These values were used to calculate state durations for wake, NREM, and REM on each day. Using these proportions and the state-partitioned metabolic profiles, we calculated the individual's total 24-hour integrated daily maintenance expenditure (IDME) under both control and treatment conditions. This value reflects the sum of each state's proportional duration multiplied by the respective state-specific maintenance costs. We then calculated the estimated treatment effects that would result if measurements were restricted to either wake only or to sleep (NREM and REM combined) only. These estimates were compared to the true integrated effect by expressing all values as a percentage of the individual's baseline 24-hour integrated SMR.