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Letter From the Editors

At its core, scientific research is a process of asking difficult questions, testing assumptions, and refining our understanding of complex phenomena. Undergraduate scholarship embodies this pursuit, reflecting the curiosity of student researchers who engage these questions through critical analysis. The *Georgetown Scientific Research Journal* provides a forum for researchers to share their scholarly findings and contribute meaningfully to academic discourse, while situating their findings within a broader context. In publishing the Spring 2026 issue, we are proud to feature work that revisits longstanding questions in the biological and social sciences and explores newer, emerging areas of scientific inquiry.

The six manuscripts featured in this issue span a diverse range of disciplines and methodologies. Three articles focus on the brain: two examine therapeutic interventions to improve neurological function following injury and disease, while a third considers ethical implications associated with brain organoids. Two manuscripts center public and global health, with one addressing maternal health disparities locally in the DMV and another that analyzes seasonal nutrition patterns in Tanzania. A sixth article extends this issue's scope beyond human populations, focusing on seasonal variation in rock hyrax behavior and energy budget. Although these studies differ in subject matter and scale, they collectively demonstrate the value of approaching scientific questions from multiple perspectives and across disciplinary boundaries.

Questions of health, behavior, and survival are shaped by biological, environmental, social, and ethical factors that rarely operate in isolation, demanding these multidisciplinary approaches. The breadth of topics represented in this issue reflects the diversity of interests among undergraduate researchers and their willingness to engage thoughtfully and creatively. We congratulate the authors on their intellectual rigor and contributions to these ongoing conversations, and we hope readers find in these pages both valuable insight and inspiration for future research.

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Implicit Bias in Maternal Health: Racial Disparities in the District of Columbia, Maryland, and Virginia Area (DMV)

Ayla Feng, Erica Hahn, Nyla Wells, Cecilia Hanuch,
Abrahim Sawez, Joan Riley, PhD

Implicit Bias in Maternal Health: Racial Disparities in the District of Columbia, Maryland, and Virginia Area (DMV)

Ayla Feng[1], Erica Hahn[1], Nyla Wells[1], Cecilia Hanuch[1], Abrahim Sawez[1], Joan Riley[2]

[1] Department of Health Care Management and Policy, Georgetown University, Washington, D.C., United States
[2] Department of Human Science, Georgetown University, Washington, D.C., United States

Email: ahf62@georgetown.edu
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Abstract

The United States has the highest maternal mortality rate among high-income countries, with disparities prevalent in the District of Columbia, Maryland, and Virginia metropolitan area (DMV). Black women experience disproportionately high rates of severe morbidity and death, persisting across socioeconomic levels, suggesting the role of implicit bias in shaping care. These biases manifest through inequitable use of interventions, stigmatized medical documentation, and dismissive provider-patient interactions, contributing to preventable adverse outcomes. Addressing these issues requires examining the presence of bias in both clinical encounters and the broader organizational, cultural, and policy contexts that perpetuate disparities. Efforts to address implicit bias must extend beyond one-time training and instead prioritize standardized protocols, workforce diversity, and structural reforms in education that lead to meaningful improvements in outcomes. Integrating policy initiatives would be a step towards reducing preventable maternal mortality among marginalized populations across the DMV.

Keywords: implicit bias, maternal health, maternal health outcomes, racial disparities

1. Scope of the Community

The United States has the highest maternal mortality rate (MMR) among the countries in the Organization for Economic Co-operation and Development.[1] Data from the U.S. Centers for Disease Control and Prevention (CDC) reveal that this high rate disproportionately affects certain populations. In 2023, non-Hispanic Black mothers experienced a pregnancy-related mortality ratio of 49.4 deaths for every 100,000 live births, compared to 14.9 among their White counterparts, indicating a significant disparity that requires attention.[2] While there are many contributing factors, this paper explores implicit bias embedded in the healthcare system and its effect on maternal health outcomes.[3] Implicit bias is an unconscious, often negative, attitude that individuals have against a specific group. It is shaped by our lived experiences and the associations we create.[4] These biases permeate the healthcare system, impacting patient-provider relationships and institutional practices.[5]

The District of Columbia, Maryland, and Virginia metropolitan area (DMV) faces maternal and infant mortality rates that are higher than the

national average and reflect inequities in access to and outcomes of care.[6] From 2018 to 2020, the fetal mortality rate in D.C. was 5.05 deaths per 1,000 live births at 24 weeks gestation, far higher than the national average of 3.67 fetal deaths per 1,000 live births. The D.C. rates of preterm and low birthweight (<2500g) were 1.5 to 2 times higher for Black mother births compared to White mothers.[7] Evidence from the Maryland Maternal Mortality Review Committee shows that, among the 18 pregnancy-related deaths that were reviewed, 83% were deemed preventable.[8] These preventable, negative outcomes disproportionately affect marginalized populations, with Black women bearing the greatest burden.[9,10] Beyond maternal mortality, preventable outcomes also include low birth weight,[11] gestational diabetes,[12] and delays in care,[13] all of which contribute to poorer maternal and infant health. This underscores the need to center obstetric care on preventing maternal death and ensuring the best possible birth outcomes.

While systematic issues, such as social determinants of health (e.g., education, income, and access to resources) may influence disparities in maternal health outcomes, pregnancy-related mortalities have been found to be higher for Black women with a college-level education than for White women with less than a high school-level education. Thus, socioeconomic differences alone cannot account for racial disparities.[14] Coupled with the preventable nature of most maternal deaths, implicit bias may be a contributor to the gaps in maternal health. More specifically, maternal morbidity experienced by Black women may be a manifestation of implicit biases and may be correlated with delayed diagnoses, inadequate treatment, and the dismissal of patient concerns, all of which contribute to higher risks of adverse outcomes such as maternal morbidity and mortality.[9]

The effects of implicit bias are experienced throughout pregnancy and the postpartum period, shaped by clinical decisions, and ultimately influences maternal health outcomes.[10] It is important to note that these outcomes stem not only from bias but also from systemic racism and structural barriers that perpetuate unequal access to quality care. Systemic racism and implicit bias are deeply intertwined within healthcare systems, each reinforcing the other. Medical doctors (MDs) display a strong preference or implicit partiality for White Americans over Black Americans, with Black MDs being the only group that demonstrated no such preference.[15] Furthermore, almost 70% of providers display implicit bias against Black or Latino patients, highlighting the urgency of the problem and the importance of further research to address implicit bias.[16] Racial patient-provider concordance may mitigate these implicit biases, yet current systems fail to provide adequate support for equitable education and advancement of Black students pursuing healthcare professions.[17,18]

2. Overview of Stakeholder Perspectives

Systemic racism surfaces as implicit bias in interpersonal situations, such as patient-provider interactions. Implicit bias often invalidates or minimizes the concerns of marginalized patients. Greater biases among healthcare providers are correlated with disparities in treatment recommendations, expectations of therapeutic bonds, pain management, and empathy.[19] These experiences can erode trust and discourage self-advocacy, ultimately worsening health outcomes.[20]

2.1 Patient Perspectives of Implicit Bias

Patient stories of their pregnancy care report that providers rushed appointments, dismissed their questions, and operated under the assumption that they lacked the capacity to make informed decisions.[21] As a result, these women reported feeling safer avoiding hospitals, since they feared the discriminatory treatment that they experienced within the healthcare system. There is a strong correlation between lower quality of care in maternal

health and implicit bias, as racial stereotypes perpetuate false narratives, such as the belief that Black women are less sensitive to pain.[3] This may contribute to racial disparities by informing medical judgments, such as counseling and offering treatment options.[22]

The effects of implicit bias are also apparent in the communication between new mothers and Neonatal Intensive Care Unit doctors and nurses. Mothers from minority communities experienced both a lower power of voice (lower levels of confidence in sharing their concerns with healthcare providers) and a lower efficacy of voice (lower responsiveness from providers to their concerns).[23] Johnson and colleagues found that physicians were 23% more dominant in conversations with Black patients than White patients, used less patient-centered communication during appointments, and conveyed a less positive emotional tone overall.[24] Black patients were also more likely to experience testimonial injustice from physicians, ranging from judgmental words to the use of quotation marks in medical records that signaled disbelief.[25] Additionally, stigmatizing language in medical records—which further perpetuates provider biases—appeared more frequently in the records of non-Hispanic Black patients than in those of their White counterparts.[26] Although these behaviors may seem inconsequential, such hindrances in bedside manner, communication, and acknowledging patient concerns can contribute to significant and potentially fatal health outcomes.

Several tragic cases illustrate this reality. Shalon Irving, an African American mother who gave birth via cesarean section, sought medical attention six different times for severe headaches and weight gain after her delivery.[27] She later died due to complications from hypertension, which nurses had noticed during previous visits, but went unchecked when the patient was told the physician was too busy. Similarly, Rodneyse Hermelyn sought obstetric care when she went into preterm labor at 22 weeks. However, the doctors deemed that they were not required to intervene to save the fetus as labor had started before the 24 weeks gestation period.[27] Ms. Hermelyn refused to terminate and was distraught over the potential loss of the pregnancy, yet the doctors sent her home, citing that they had other patients to attend to.[27] Though she eventually found proper care elsewhere at a clinic primarily serving low-income patients, those who dismissed her case jeopardized her physical and emotional health.[27]

Pregnant Black women often feel devalued in health clinics and unwelcome in social services, burdened by racial assumptions and the need to prove themselves in order to be treated with respect.[28] Some Black women felt that such racialized stigmas contributed to psychological stress and reported that these experiences made it difficult for them to have a healthy pregnancy.[28] Emerging evidence has similarly indicated that prenatal maternal stress is a significant contributor to adverse birth outcomes.[29,30] Black mothers who experienced racial discrimination on three or more instances during pregnancy had a 3.1 times higher risk of preterm delivery and were 2.4 times more likely to give birth to infants with low birth weights than their White counterparts.[31] Even after adjusting for smoking, alcohol consumption, and depressive symptoms during pregnancy, this disparity was not reconciled.[31]

2.2 Provider Biases in Clinical Practice

Implicit bias can be apparent in clinical practices from the treatment plans recommended by providers to the screenings and tests that pregnant women undergo. Evidence suggests that cesarean delivery, particularly primary cesarean, significantly increases maternal mortality risk compared to unassisted vaginal delivery.[32] Black women are more likely to experience pressure to undergo cesarean delivery and less likely to have their preferences respected during childbirth.[33] In addition, pregnant

women with Black racial identity experienced more non-consented procedures during perinatal care and vaginal births than those identifying as White, and women who identified as other minoritized races and ethnicities experienced greater pressure to accept perinatal procedures compared to White women.[34]

Black patients have also been disproportionately subjected to urine toxicology testing during labor and delivery, regardless of their history of substance use.[35] Black patients did not have a higher probability of a positive test result than other racial groups. These procedural inequities operate within broader social structures that have historically assumed harmful, race-biased health behaviors among racial and ethnic minorities, thereby demonstrating the effects of implicit bias in clinical decision-making.

Many providers lack cultural competence and awareness of racial health disparities, and these deficiencies could allow implicit biases to persist unchallenged.[36] Black healthcare providers have often been marginalized and dismissed from careers in obstetrics, encouraging a cycle of negative narratives against Black mothers and underrepresentation of those who could offer more culturally responsive care.[21] Black mothers who developed supportive relationships with healthcare providers felt more comfortable sharing important health information and discussing their concerns.[28] Increasing minority representation and education in healthcare fields are therefore essential steps toward reducing implicit bias in healthcare and improving the postpartum care of Black women. However, these goals remain difficult to achieve in environments of systemic oppression and provider barriers.

3. Potential Solutions and Resources

These findings highlight the importance of training providers to recognize implicit bias and to address the structural barriers in healthcare that uphold these prejudices. There are many resources, successful practices, and policies in place to meet these needs in the DMV, yet these unequal practices persist.

3.1 Local Initiatives

Community-based organizations have stepped into action to mitigate some disparities involved with access to care, especially for D.C. residents in low-income and predominantly Black neighborhoods in Ward 8, who travel nearly three times the distance of other residents to reach hospitals offering labor and delivery services.[37] For example, the Community of Hope's Family Health and Birth Center and Mary's Center serve uninsured patients in D.C. Wards 7 and 8. These centers provide comprehensive, team-based prenatal care that integrates midwives, doulas, and social workers who share cultural and community connections with the patients.[38,39] This approach of integrating community partnerships could improve outcomes for Black mothers because it builds trust, continuity, and advocacy during labor by providing culturally appropriate care.[40,41] In addition, integrating social workers for transportation and incorporating Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) referrals helps address the health disparities rooted in structural racism and the systems that sustain it. Addressing these structural barriers is key to addressing the roots of implicit bias.[38,39]

3.2 Policies Addressing Implicit Bias

In Maryland, legislation on a Structural Racism Bill, HB 783, which would require an implicit bias training program approved by the State Health Occupations Board for all healthcare professionals in 2026, has been recently approved.[42] Virginia had a similar bill in 2023 that was vetoed, but an effort to revive the legislation is anticipated under the newly elected governor.[43] Mandated

training by State Occupational Boards for licensure is the first step to raise awareness of the problem.

The D.C. Maternal Mortality Review Committee recommended that the Department of Health require healthcare professionals to complete implicit bias and cultural bias training.[44] Building on these recommendations, the D.C. Perinatal Quality Collaborative implemented implicit bias training across major birthing hospitals, including Howard University Hospital, Medstar Washington Hospital Center, and Medstar Georgetown University Hospital.[44] These bills mandate that licensed healthcare providers must receive training on implicit bias, a first step to increasing knowledge and awareness of the problem. The D.C. Department of Health also offers resources for healthcare organizations to incorporate into their programs, such as the Implicit Association Test and a few debiasing strategies to help communities work toward more equitable practices.[45] In addition, the *D.C. Values in Action* initiative aims to build a culture where all D.C residents feel and are safe.[46] Health systems, such as MedStar in the DMV, have hotlines and opportunities to report experiences of bias or discrimination.[47,48]

4. Current Gaps and Future Outlooks

Despite these advances and the resources available, there remain gaps in evidence and implementation. The implicit bias trainings that many hospitals adopt are one-time workshops that aim to improve awareness. Ultimately, these trainings fail to address the deeper behavioral and structural aspects of implicit bias, and they lack tangible outcome measures for evaluation.

Mishkin and Flax found that one workshop had increased participant knowledge of bias from 62.5% to 83.8%.[49] This workshop also increased anecdotal observations by staff, where they were able to identify implicit bias in real time more readily than before the trainings.[50] However, these initiatives do not provide meaningful evidence of improved clinical decision-making and patient outcomes in target populations.

While the current implicit bias training increases basic knowledge and awareness, lasting behavioral and outcome change depend on integrating standardized decision protocols, race-stratified data analysis, and equity-focused quality improvement programming.[51] For example, Brazil's Abraço de Mãe program that integrates anti-racist practice into its pre-existing quality improvement training found a 34.2% decrease in maternal deaths within two years, from 83.7 to 55.0 deaths per 100,000 live births.[52] They fully engaged the staff through transparent data-sharing: stratifying hospital data by race and allowing them to observe patterns and identify any disparities to revise the care protocols accordingly.

Initiatives such as open note-taking and making physician notes visible to patients would encourage transparency, possibly mitigating implicit bias in care.[3] In addition, standardization of decision points through reliable algorithms instead of pure physician subjective discretion was seen to minimize biased judgment in care delivery.[36] Optimization of organizational practices is also an area of extreme importance in mitigating implicit bias in maternal healthcare. Implicit bias training evaluations frequently measure provider satisfaction or self-reported awareness rather than objective maternal health outcomes and indicators.[51,52] Having concrete, outcome-based metrics is critical, as it allows researchers and hospitals to develop and tailor more effective training that yields improvements in care quality and outcomes.

5. Conclusion

Successfully addressing implicit bias requires more than designing appropriate training methods; it needs structural reform to medical education and the healthcare system. Implicit bias is reinforced

through the curricula, faculty composition and attitudes, and institutional culture.[53] Evidence shows that faculty role models of color and diverse learning environments reduce racial bias among medical students.[54] There should be more efforts to change the foundation of professional formation by integrating antiracist pedagogy and representation. Beyond medical education, Green and colleagues highlight the importance of workforce diversity in healthcare, staff treatment and retention initiatives, and clinician psychological support in a healthy, equitable hospital environment.[50] Without these systemic changes, efforts to address implicit bias will remain limited, leaving the structural conditions that perpetuate inequitable maternal care largely unchanged.

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Brain Organoids – Sentience, Consciousness, Personhood, Agency, and Moral Status in Neuronal Cell Cultures

Benjamin Cohen

Brain Organoids – Sentience, Consciousness, Personhood, Agency, and Moral Status in Neuronal Cell Cultures

Benjamin Cohen[1]

[1]Department of Neurobiology, Georgetown University, Washington, D.C., United States

Email: bdc64@georgetown.edu
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Abstract

Recent advances in the use of induced pluripotent stem cells and culture media that mimic the three-dimensional structure of the extracellular matrix have permitted the development of human brain organoids for *in vitro* disease modeling. However, organoids present a highly salient ethical risk, with significant concerns regarding potential consciousness, moral status, and legal personhood contingent upon sentience, as well as socioeconomic barriers to access. This article explores the challenges associated with the application of brain-based consciousness indices to organoids, which are currently incapable of communicating mental states and possess a unique functional organization. While recent philosophical shifts associated with the rise of artificial intelligence (AI) have led to expansive definitions of consciousness and agency, applying analogous paradigms to organoids overlooks ongoing harms to model organisms used in biomedical research and risks delaying neurological drug development, exposing human trial participants to side effects, and discarding promising therapeutics before they reach the clinical stage. Neuroethical frameworks surrounding organoids must avoid distraction by highly speculative apprehensions, and instead emphasize practical issues of informed consent in sample collection and unrestricted entry to minimize the potential harms for patients and researchers.

Keywords: human brain organoids, neuroethics, theories of consciousness, sentience, agency, personhood, moral status

1. Disease Modeling Without the Model Organism

After months of progressive weakness and cognitive decline, a battery of standard neurological tests brings no answers for a previously healthy patient, ruling out dementia, slow-moving infections, and rare autoimmune conditions. In a desperate attempt to uncover the diagnosis, geneticists turn to whole-exome sequencing, a technique that reveals multiple unexplored mutations affecting the prion protein.[1] The prognosis is terminal, yet the novel disease becomes a tool for unveiling the mechanisms of prion-related neurodegeneration. Drawing a cell line with the mutation of interest from the patient, researchers generate a three-dimensional culture that measures only millimeters in diameter. The approach incorporates regional differences in brain organization and interactions between neurons and glia, providing insights into prion disease through RNA sequencing and cellular imaging.[2]

While the routine use of personalized *in vitro* disease models for patients with undiagnosed

neurological conditions remains distant, human brain organoids that contain neurons in a three-dimensional framework mimicking the mechanical properties and protein composition of the extracellular matrix alongside other central nervous system cell types, such as astrocytes, oligodendrocytes, and microglia, have already been engineered to study a wide range of disorders, including Alzheimer's disease, amyotrophic lateral sclerosis (ALS), schizophrenia, and post-COVID-19 neuroinflammation.[2-4] Organoids offer a distinct advantage over two-dimensional human neuronal cell cultures and animal brains, allowing developmental signaling molecules to better establish spatial gradients while permitting direct translation of results to treat patients and mitigating the potential for failure when new drugs are advanced from preclinical animal models into human trials.[3,4] However, the process of generating organoids brings both compelling benefits and troubling ethical considerations. Organoids originate from human-derived stem cells, ensuring a genome identical to the patient whose disorder is being investigated, and reducing the need for animal models that may imperfectly approximate diseases.[4,5]

For neurological conditions that defy a precise, single-gene explanation, stem from mutations at functionally ambiguous sites, or involve unknown epigenetic changes, organoids can be invaluable for understanding the mechanisms that drive symptoms. Methods ordinarily infeasible in live human tissue could be applied to organoids, including electrophysiology, scRNA-seq, a technique to measure gene expression at the transcriptional level in individual cells, and ChIP-seq, which involves sequencing sites in the genome bound by a particular protein to identify abnormal interactions with DNA.[6] Nonetheless, the brain-like nature of organoids generated from human biological material raises significant and visceral fears, as the technology could represent a *functional analog* for the human brain. With sufficient complexity and synapse count, the ability of organoids to recapitulate the cellular composition and organization of neural tissue may enable the development of circuits and activity patterns resembling those found in the human brain. Consequently, the neuronal cell cultures could model a human range of cognitive characteristics and acquire the capacity to respond to the environment, vulnerability to moral harm, a sense of "self," or even consciousness.

Organoids represent effective and biologically similar models of human brain disorders, but fears that the neuronal cell cultures are conscious may give pause to widespread implementation in research. As its central argument, this paper aims to demonstrate that such concerns are fundamentally speculative in nature; organoid consciousness is difficult to verify empirically, and distant when weighed against the practical implications of improving human health through research and reducing the suffering of laboratory animals. Overstating the possibility for consciousness threatens to shift the focus away from the scientific benefits and more imminent ethical issues associated with neuroscience research in organoids while also creating regulatory hurdles.

2. The Anatomy of Consciousness

The perception that organoids are full-fledged microcosms of the human brain has become pervasive, largely due to the sensationalist headlines of popular science publications. "Minibrain" has turned into a widely-used buzzword for organoids, and while it effectively communicates their role as human brain models, the term creates an illusion of a functionally complete organ, fueling misconceptions and overestimations of the cultures' capacity for information processing and humanlike abilities.[7-10] Organoids are typically restricted to a single brain region; forebrain models have been generated to investigate Zika virus-induced

microcephaly and midbrain models to study Parkinson's.[11] In the philosophical sense, consciousness remains a topic of continuing debate, but is generally considered to involve first-person and subjective elements; in a conscious cognitive or perceptual experience, there is "something it is like" for the subject that is having a feeling.[12,13] Neuroscience, for its part, dodges the challenge of providing a mechanistic explanation or account for how consciousness arises, instead either correlating it with certain patterns of neural activity based on the observed and reported experiences of patients, or going a step further and establishing a direct identity relation with neuronal firing.[14] Neuroscientific theories of consciousness are primarily designed for use as diagnostic tools in unresponsive, brain-injured patients, not as complete explanatory paradigms. Thus, they may place varying levels of emphasis on certain aspects of brain activity. For subsequent discussion, the working interpretation of consciousness will draw on both philosophical and neuroscientific conceptions, where consciousness is a first-person subjective phenomenon associated with specific changes to physical states in the neurons of the experiencer, which are detectable but do not provide a causal account. However, even with such a composite definition, the notion of consciousness remains somewhat too broad and murky to address the central moral challenge of whether it is acceptable to subject organoids to experiences that might be painful or restrict their flourishing.

From an anatomical standpoint, consciousness requires the presence of a system that is able to parse and integrate large consciousness requires the presence of a system that is able to parse and integrate large amounts of information from multiple, functionally-distinct brain structures into an experience.[15] Such a condition for consciousness is consistent with biological intuitions. For example, while the number of neurons in the human spinal cord or gastrointestinal tract may rival that of an insect or small mammal brain, we tend not to situate consciousness in those divisions of the nervous system because they lack an identifiable center for the convergence and processing of the information contained within neuronal firing. It then follows that a single-region organoid lacks the basis for consciousness—recapitulating the properties of only one brain area, it has neither a subspecialized, information-integrating system, nor inputs from multiple functionally distinct regions. While attributing consciousness to a particular brain structure or characteristic may be counterproductive or excessively reductionist, the *thalamus* is known to facilitate the multisensory integration of information through reciprocal signaling loops with the cerebral cortex, and empirical evidence from intraoperative neurostimulation on human patients suggests that the *claustrum* or *left anterior insula* may be required for consciousness.[15,16] By definition, there cannot be a "thalamus-like" or "claustrum-like" specialization within a neuronal cell culture that is functionally homogeneous, which would appear to shut the door on consciousness for single-region organoids.

Whole-brain organoids contain multiple regions corresponding to the midbrain, forebrain, spinal cord, and other broad divisions of the central nervous system, and may present more compelling cases for potential consciousness due to greater heterogeneity in cell types and circuits.[17] However, all organoids currently remain limited to the millimeter scale, as inadequate vascular development starves cells of oxygen and leads to necrosis at the center of the structure, which experiences hypoxia due to a lack of direct contact with culture media.[3] While brain size is not directly correlated with capacity for consciousness, a critical quantity of cerebral cortex is likely required for the necessary information-integrating ability; even the largest organoids are smaller than mouse brains by an order of magnitude, and may fail to meet such a benchmark.[18]

3. Adjudicating the Status of Organoids Based on Sentience

The judgment about personhood, agency, and moral status in organoids is ultimately contingent upon *sentience*, with consciousness as the first prerequisite. It is instructive to turn towards the concept of sentience, defined here as the capacity for valenced experiences—those that feel good or bad, with the potential to provoke or avert suffering.[19] *Suffering* refers to any *negatively valenced experience* that a subject desires to avoid, regardless of whether its basis is physical, psychological, or both.[20] Sentience is therefore predicated upon consciousness, as it implies *having an experience*, although the two concepts should not be considered coextensive. Neural activity could generate "white noise" feelings that are *conscious experiences* in the sense of "being like something," but lack the motivational or valenced component of *sentience,* particularly in the earliest-diverging phylogenetic groups of animals with rudimentary nervous systems analogous to small and structurally simple organoids.[21] However, as organoids cannot communicate potential experiences of suffering and happiness to an experimenter, it is impossible to directly measure their *capacity for sentience*, making it necessary to draw on an evaluation of the conduciveness of their neural architecture to *consciousness*. Even if organoids can be shown to possess biological hardware consistent with consciousness, such an argument will have only accomplished the minimal condition to begin staking the claim of sentience. The consciousness-sentience chasm bears relevance to the later appraisal of speculativeness in the ethical calculus of organoids, and must be considered when analyzing the prospect of organoid consciousness.

4. Biological Theories of Consciousness

Several frameworks offer biological explanations for consciousness, supported by varying degrees of plausibility and empirical validation. The *Orchestrated Objective Reduction* theory situates consciousness in quantum coherence between neuronal microtubules, but fails to account for the role of neurotransmitters, receptors, and postsynaptic potentials, providing no clear avenue for experimental observation.[22] The *Global Workspace* theory posits that incoming data can generate a conscious experience when it enters the "global workspace" of distant brain regions connected by long-range projections, allowing the information to be simultaneously utilized by several specialized areas.[14,15] The *Integrated Information* theory assigns a φ *(phi)* value to measure the amount of information, or reduction of uncertainty, generated in an integrated matter by circuits and systems that cannot be separated into independently functioning submodules. While the mathematical definition of φ is complex, it can be approximated without data from individual neurons through measures of brain activity, such as electroencephalogram (EEG) or functional magnetic resonance imaging (fMRI).[14,15,23,24] Both the *Global Workspace* and *Integrated Information* theory suffer from a pivotal flaw—they do not provide a deep, causal explanation of how patterns of neural activity produce consciousness, and instead focus on characteristics of the entire system, while not accounting for small-scale brain processes. Neurostimulation studies face a similar issue, with the added concern of limited sample sizes that may fail to account for variability among individuals. Additionally, both theories overgeneralize the concept of consciousness to systems that are inconsistent with intuition. The *Global Workspace* theory posits that a computer system made up of multiple interconnected modules is conscious.[25] The *Integrated Information* theory goes even further, suggesting a single, independently functioning light sensor is conscious, simply because it generates information beyond what is contained in its components alone.[25]

5. Challenges With Measures of Consciousness

While neuroscientific theories may offer specific metrics of consciousness, they can at best provide a surface-level predictive account of select biological aspects of experience. Consciousness measures are inherently correlational and hindered by an "explanatory gap"; even if a particular theory perfectly predicts consciousness when some value *C* is greater than 100, it is not clear why or how C > 100 has anything to do with first-person subjective conscious experience.[14,26] The clinical application of the *Integrated Information* theory, for example, relies on a perturbational complexity index (PCI), an analog for φ that evaluates EEG responses to exogenous magnetic or electrical stimulation to measure consciousness.[27] However, it would be unreasonable to assume that a certain PCI value is identical or absolutely correlated with the experience of consciousness. Although the multiple realizability of consciousness is somewhat limited by the requirements of sensory systems and brain structures, conscious experiences could still arise through a vast but finite set of possible neural activity patterns, with many producing drastically different values of PCI or other indices.[28] In a speculative study predicting recovery among patients in vegetative states, PCI proved inaccurate in 1 of 8 cases, signifying that consciousness indices are often imperfect and unreliable.[27]

Humans represent the easiest model with respect to detecting consciousness, as they are capable of verbally reporting experiences, enabling researchers to clearly establish correlations between neural and mental events. The problem of identifying consciousness in organoids is infinitely more vexing. Without clearly observable behavior, theories and indices are necessary to determine whether an organoid is conscious, yet such measures were initially developed and validated using human studies. If a mental state can be produced by several patterns of activity even when considering the brain of a single individual, it stands to reason that the divergence of underlying neural firing could be far greater between a human and organoid undergoing the same experience. Direct observation of an organoid's neural activity using an electrode array is possible, but unlikely to be fruitful. Even if the precise state of every neuron is continuously measured—which would require a degree of spatial resolution that is currently unattainable—there is no way to definitively assign meaning or function to the firing since the neural code of each cell in the organoid is unknown.[29] Biological computing platforms integrate organoids with traditional hardware and harness activity for information processing, suggesting that meaning may be assigned to neural activity without human-interpretable communication on the part of the organoid. Such systems are based on closed-loop stimulation, where sensory inputs in electrical form are supplied by a researcher according to the organoid's outputs, enabling learning through changes in synaptic strength.[30] While the organoid is not entirely passive, correlations between neural signatures and organoid-generated behavioral events, like moving the paddle in a video game of Pong, are preset by the experimenter.[30] An organoid lacking sensory stimulation is a black box of spontaneous neural activity, without any human-determined, external frame of reference to attribute meaning. In practical terms, these limitations of identifying organoid consciousness suggest that quantitative metrics are predictors, not proxies, for consciousness in the pragmatic and phenomenological sense. Numerical measures of consciousness can at best represent theories for the evaluation of an ethical risk, rather than means to reaching definitive conclusions, with waning effectiveness when applied beyond the organisms in which they were originally developed.[30]

6. Scientific Benefits of Organoids

Organoid consciousness cannot be entirely excluded, despite anatomical characteristics and activity indices demonstrating its low probability,

precisely on account of the difficulties in translating metrics from humans to other systems. Nonetheless, it is essential to evaluate the potential for moral harm in relative terms; organoids carry only a remote possibility of suffering when terminated in comparison to animal models, which are routinely subjected to harmful protocols.[31] When the prospect of applying research to relieve human suffering is distant in comparison to the harm inflicted on animals, organoids can sidestep the additional question of whether the experimentation is *ethically excusable*.[32] In fact, the very existence of animal experimentation assumes that model organisms must be *fundamentally similar to Homo sapiens* for meaningful clinical application of the findings, signaling that humanlike suffering is occurring on a grand scale.[32] Even if there is some likelihood for consciousness in organoids, any claim of suffering would add a further layer of improbability through the requirement for *sentience*, and the corresponding assertion that their experiences not only exist but have valence. On the other hand, many forms of animal experimentation inherently assume that suffering is occurring in order to be valid; established paradigms in neuroscience and psychology, such as the forced-swim test for rodents, are explicitly designed to model and evaluate suffering from depression.[33] The existence and severity of animal suffering strikes at the central issue with precautionary arguments surrounding the use of organoids in research. While it may be an instructive exercise to "err on the liberal side" regarding the attribution of consciousness to organoids, doing so threatens to downplay the possibility that applying the neural cell culture technology in research can prevent ongoing, obvious harm to animals.[34] If science makes an effort to shift as much animal experimentation as possible into organoids, and they are later determined not to suffer, a great deal of harm would have been prevented. In the unlikely event that organoids do turn out to suffer, the outcome will be a "moral tie," for the suffering will have been shifted from one category of subject to another. While one might attempt to assert that suffering is ethically worse when experienced by an organoid compared to an animal, the claim would be tantamount to a speciesist argument that the human origins of the organoid's cells make it morally superior.[32]

Beyond the definitive relief of animal suffering, organoids have the unique potential to *reduce or prevent human suffering* in ways that animal models cannot. New drug candidates for neurological diseases can be screened with much higher throughput in organoids compared to model organisms, which reduces the time to find an effective hit and begin clinical trials, ultimately improving and saving more human lives.[35] Furthermore, brain organoids could be generated from a patient's own cells and auto-transplanted for reconstructive purposes to treat strokes and traumatic injuries.[35] For reasons that are often poorly understood, animals or other models may fail to accurately replicate uniquely human biology, leading to unexpected and severe side effects. The trial of TGN1412, a novel drug designed to suppress the immune system, is a prominent case in point; after successful testing in monkeys, human volunteers received the compound, only to experience the contradictory effect of extreme immune activation, leading to multi-organ failure and hospitalization.[36] While more easily overlooked, organoids also bring the unique advantage of minimizing the number of "missed" new pharmaceuticals, which may be safe and effective in humans or human-derived cells, but are abandoned before reaching clinical trials due to harm or lack of effect in animals.[36]

The applications of *Organoid Intelligence (OI)* to biological computing could bring further benefits to humans in the clinical sphere and beyond. Connecting organoids to conventional computer hardware could enable information processing in a manner analogous to AI and machine learning, only far more energy-efficient.[37] The resulting organoid intelligence systems could ultimately be used to

analyze large amounts of biological data for diagnostic and drug development purposes and may even take over the role of conventional computing hardware in the longer term, preventing human suffering from environmental contamination associated with excessive and irresponsible energy use.

7. Agency, Legal Personhood, and Moral Status

Aside from hindering the use of organoids, centering speculative ethical concerns can invite misunderstandings that may lead to counterproductive regulation. Terminological confusion regarding organoids extends to the concept of agency, driving fears that could unnecessarily hinder drug-development efforts for neurological diseases. Established theories of agency involve intentional action, making it difficult to classify organoids as agents without ascribing goal-oriented, motivated mental states, which would require the implausible classification of organoids as *sentient* when *consciousness* alone is already quite unlikely.[38] Under recent reconceptualizations within the philosophy of technology, an *agent* must merely be distinct from its environment with the capability for behaviors that occur without external causation and adaptive responses when inputs change.[39,40] While such frameworks are useful for highlighting the promises and pitfalls of AI by attributing agency to novel technology, they lead to frivolous and unhelpful categorizations elsewhere. If an organoid could be classified as an agent, so could a bacterium, or even a large macromolecule capable of self-assembly. Applying an excessively broad definition of agency risks confusion with the more restrictive and consequential notion in widespread use, leading to peculiar limitations on organoid research, which might become formalized through the concept of *legal personhood.* If animals that display outward signs of consciousness, such as an orangutan in Argentina and an elephant in Pakistan, have been accorded rights and protections under the subcategory of *natural persons,* which encompasses the non-corporate legal category of born, biological individuals, what prevents courts from granting the same status and protections to organoids on precautionary grounds?[41,42] Legal frameworks for personhood might be expanded to encompass organoids based on the assumption that the neuronal cell cultures have the capacity for complex responses or internal mental states comparable to species like hippopotamuses, dolphins, and dogs.

Assigning personhood to organoids would also lead to the attribution of *moral status* as a consequence, which requires that a subject has interests that matter for its own existence.[43] While the boundaries of legal personhood vary by jurisdiction, it has so far been limited to humans and animals that display evidence of complex, goal-directed actions, suggesting that the category necessarily implies moral status. By consequence, legal personhood for organoids would either imply that a subject can have rights without clear evidence of interests, which appears logically inconsistent, or that the neuronal cell cultures have *moral status*, and, thus, its prerequisite condition of *sentience.*[44]

Therefore, attributing legal personhood to organoids would not only create a regulatory hindrance, but also inadvertently hand down an ontological judgment on whether organoids are conscious by statute alone. If organoids are nonconscious entities devoid of interests and cares, it follows that they lack *moral status in themselves*, making their destruction after use in research seemingly inconsequential. However, the act can still bear ethical implications by way of emotional suffering for other subjects, including family members, despite not directly affecting patients, such as in the case of unauthorized collection of body parts from deceased individuals at the Alder Hey hospital in Britain.[45]

Granting organoids legal personhood could open up avenues to the sanction or criminal

prosecution of researchers for inflicting harm on organoids, posing a significant hindrance to studies with clinical applications. Currently, the use of animals in research in the United States is guided by the *Improved Standards for Laboratory Animals Act* of 1985, which requires the establishment of an Institutional Animal Care and Use Committee (IACUC) that provides internal oversight for animal research.[46] However, the IACUC is not mandated to report to the USDA for enforcement by inspections and investigations, leaving a legal loophole for unnecessary or harmful animal research to continue.[47] While the Act is explicitly limited to warm-blooded research species, does not grant personhood, and excludes *in vitro* models like organoids, increasing awareness of complex animal cognition could conceivably lead to changes that make classifications based on broader characteristics, such as the ability to intentionally respond to stimuli.[47] In that event, the legal structure for animals could potentially be applied to organoids, stifling researchers with investigations and fines in the absence of conclusive evidence for animal-level cognition in organoids.

A similar issue surrounding the legal classification of organoids could arise through regulations designed to safeguard patients in clinical trials. The 2018 revision of the Common Rule of the Code of Federal Regulations defines a *human research subject* as "a living individual about whom an investigator conducting research obtains information or biospecimens," making the inclusion of organoids under clinical trial protections dependent upon their categorization.[48] Considering the common tendency to situate the essence of a person in the brain and mind, organoids that develop sufficiently humanlike cognitive abilities might be categorized as "living individuals" in a regulatory context, prompting interpretations of the Common Rule that could prevent research institutions from receiving federal funding, or mandate that organoids be granted data confidentiality and provide informed consent for any procedures or compounds administered. The bipartisan FDA Modernization Act 2.0 of 2022 represents a positive step by doing away with requirements for preclinical animal models and explicitly permitting "cell-based assays" and "other nonhuman or human biology-based test methods," which would encompass organoids.[49] Nevertheless, it remains important for state and local regulations, as well as laws in other nations, to avoid restricting the use of organoids in research through overly-broad or ambiguous applications of terminology.

8. Prioritizing Utility and Balance

Beyond hindering or reducing the use of organoids altogether, ethical fears surrounding consciousness distract from more imminent disparities in access to the technology. Gene editing and RNA interference-based therapies are already in development for a range of heritable neurological conditions, including Huntington's disease and epilepsy. As a result, organoids are not only research tools, but practical platforms to study rare neurological conditions in order to develop custom treatments targeted to a patient's specific mutation.[50] However, the resources necessary to produce organoids are significant; reagents alone can cost up to $5,000 per organoid, in addition to lab equipment and labor.[51] While clinical trials typically provide free investigational drugs, individualized therapeutics requiring organoids for testing could limit access to patients with adequate financial resources and nations with universal healthcare systems that cover expensive rare disease treatments, exacerbating socioeconomic disparities once commercialized. For individuals with extremely rare diseases, testing a potential treatment in a typical randomized controlled trial may be infeasible due to a small affected population, making an evaluation in a preclinical model followed by a single-patient approval through the FDA's expanded access pathway the only possible option.[52] In an era of

personalized medicine, patients who lack the resources to have prospective treatments tested in organoids generated from their own cells for high-quality preclinical data might face greater uncertainty and an increased risk for side effects. Poorly conceived applications of organoids also threaten to siphon attention away from biomedical research. The case of the American composer Alvin Lucier, who donated blood to produce organoids that generate music through spontaneous activity after his death, raises a unique question of whether serving as the cell donor for an organoid can justify copyright over the creative results of its neural activity. Such experimentation may be considered wasteful in utilitarian terms, taking finite resources away from research and clinical studies.[53]

The practical consideration of informed consent in the harvesting of patient samples for research deserves special attention for organoids. While biosample collection is already tainted by the ethical issue that material can be obtained from an unconscious patient without their knowledge, analogous to HeLa cells harvested without consent, organoids invite further questions surrounding patients' decisional capacity. Since brain organoids are used to model neurological disorders that may produce a cognitive decline in the patients from which cells are derived, determining whether a subject truly comprehends the implications of participating in a study may extend beyond signatures on a form, requiring a multidisciplinary evaluation. While ethical scholarship surrounding neurological patients has understandably focused on issues of clinical care, it would be prudent to adopt similar measures of advance directive and power of attorney in relation to the contribution of cells and genetic material for research use.[54] Considering the broad success of organ donor registration with driver's license applications to improve accessibility to timely transplants, a similar measure of advance consent could be implemented to collect tissue samples that might be used to generate organoids.

9. Precautionary Arguments

The case of organoids also prompts broader examination of the scenarios under which precautionary arguments surrounding new technologies are more or less effectively applied. In scenarios such as germline genome editing, the harms posed by inaction compared to action are asymmetric; the severity of the "worst-case scenario" is both uncertain and extremely high. For example, not performing germline genome editing leads to known suffering in patients born with genetic disorders, while doing so comes with a low probability of highly detrimental outcomes, such as off-target effects leading to more disabling conditions, or creating novel forms of discrimination based on individuals' edited status. However, the reckoning is markedly different for organoids. The exclusion of organoids from research will cause suffering in animal models and humans with certainty, while the analogous consequence is more remote if organoids have their niche in research. Unlike with genome editing, the harms of action versus inaction are *symmetric* for organoids; in both cases, living subjects experience a similar manner and extent of suffering, for it would be challenging to claim that the negatively valenced experience of an organoid is worse than that of an animal. It therefore appears that asymmetry and high uncertainty in possible harms are key factors in determining whether precautionary arguments are sensible.

10. Conclusion

The approach to human brain organoids must strike a judicious equilibrium, aiming to maximize the benefit for patients with neurological conditions and prevent the most imminent ethical risks of informed consent and societal disparities. Moral concerns associated with remote possibilities of organoid consciousness and suffering must be appropriately weighed against the ongoing harms

inflicted upon animals and humans as a result of biomedical research. Unless proven to possess mental states, caution regarding consciousness and moral status must be exercised through neurobiological, philosophical, and legal optics with the terminologically clear assumption that *organoids are a technology without sentience or personhood*, and adequate attention must be directed towards commonplace ethical challenges rather than speculative concerns.

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Seasonal Fruit and Vegetable Intake Among Semi-Urban and Rural Households: A Secondary Analysis from the 2012/2013 Magu HDSS, Northwestern Tanzania

Fritze Mayer, Jacqueline Materu, Antidius Rwehumbiza, Denna Michael

Seasonal Fruit and Vegetable Intake Among Semi-Urban and Rural Households: A Secondary Analysis from the 2012/2013 Magu HDSS, Northwestern Tanzania

Fritze Mayer[1], Jacqueline Materu[2], Antidius Rwehumbiza[2], Denna Michael[2]

[1]Department of Global Health, Georgetown University, Washington, D.C., United States
[2]Mwanza Centre, National Institute for Medical Research, Mwanza, Tanzania

Email: cfm75@georgetown.edu
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Abstract

From 2013 to 2023, over 90% of the population in Tanzania fell below the WHO-recommended fruit and vegetable (FAV) intake of ≥5 servings/day. Variation between wet and dry seasons further influences dietary intake and stability, which has implications for nutritional adequacy and non-communicable disease (NCD) risk, especially in rural areas. However, evidence on seasonal FAV intake and its determinants in Northwestern Tanzania is limited. This study aimed to describe the adequacy and seasonality of FAV intake and examine associations with socio-demographic and health factors in Northwestern Tanzania. Data were collected from 2012-2013 as part of regular serological surveys in the Magu Health and Demographic Surveillance System (HDSS), including 7,620 participants. Three multinomial logistic regression models were used to assess associations between seasonal FAV intake and socio-demographic and health characteristics. Most participants (96.1%) reported inadequate intake in both seasons. FAV adequacy and intake measures were significantly higher in the wet season compared to the dry season. In adjusted analysis, females and overweight/obese individuals had a lower relative risk of inadequate intake, whereas rural residents and adults aged 50 years or older had a higher risk. These findings emphasize a need for further research and interventions incorporating seasonal variation on FAV intake, with a focus on vulnerable groups and improving year-round accessibility of culturally relevant, affordable FAV within local environmental contexts.

Keywords: fruit and vegetables, seasonality, healthy diet, Tanzania, Magu HDSS

1. Introduction

Tanzania, like many Sub-Saharan African (SSA) countries, is experiencing a rise in the burden of non-communicable diseases (NCDs).[1] Concurrently, the country faces a triple burden of malnutrition, characterized by persistent stunting, increasing rates of obesity, and widespread micronutrient deficiencies.[2,3] These health challenges are driven by multiple factors, including low dietary diversity, rapid population growth, urbanization, and shifts in physical activity patterns and food preferences.[4,5] As diet is a critical determinant of nutrition and disease outcomes, understanding patterns and inadequacies in food consumption is essential for improving population health and reducing disease burden.[2]

One key component of a healthy diet is fruit and vegetable (FAV) intake. Fruits and vegetables provide essential vitamins, minerals, phytonutrients, and dietary fiber, which support overall immune and metabolic health.[7-10] Furthermore, sufficient FAV intake is associated with reduced risk of many NCDs, including cardiovascular disease, type 2 diabetes, and hypertension, as well as all-cause mortality and micronutrient deficiencies.[8,11-15.] These benefits are maximized when individuals consume at least 5 servings, or approximately 400g, of fruits and vegetables daily.[12,6]

Despite these well-documented benefits, FAV intake remains inadequate worldwide. In 2021, approximately 2.6 million deaths were attributable to suboptimal FAV intake.[16.] Only 80% of adults in low- and middle-income countries (LMICs) consume >5 servings/day.[17] In Tanzania, over 90% of the population did not meet this standard in 2013 or 2023, as reported by the STEPwise approach to Surveillance (STEPS) surveys, which assess NCD risk factors at the population level.[18,19]

The Tanzanian government has recognized nutrition and dietary diversity as public health priorities. The National Multisectoral Nutrition Action Plan II for 2021–2026 outlines strategic actions to reduce malnutrition, including a target to lower the prevalence of inadequate FAV intake to 68% by 2025–2026.[2] Similarly, the National Strategic Plan for Prevention and Control of Non-Communicable Diseases (2021–2026) emphasizes dietary awareness and FAV intake.[1] Together, these documents reflect government commitment to FAV intake as an indicator of diet quality and population health, yet data suggest this priority has not translated into practice, as inadequate consumption remains widespread. FAV is a particularly relevant indicator in Tanzania, as it reflects diet quality and micronutrient adequacy, but is strongly constrained by affordability, availability, and seasonal agricultural cycles. Moreover, FAV intake is a globally recognized indicator, allowing comparability across countries and over time. Despite national-level monitoring, there remains a limited understanding of how FAV intake varies across socio-demographic groups and environmental conditions.

Dietary stability refers to the ability of individuals and households to maintain dietary adequacy over time despite environmental or economic fluctuations.[20] Dietary diversity, on the other hand, reflects the variety of foods consumed at a single point in time. Hence, individuals may have diverse diets in one season but still experience dietary instability. In Tanzania, seasonality is an important part of dietary stability, particularly in rural settings where livelihoods and food access are closely related to agricultural cycles.[21] Variations between wet and dry seasons affect the availability, variety, and cost of foods, especially for fruits and vegetables, leading to fluctuations in diet quality and a risk of micronutrient deficiencies.[22-24] Evidence from SSA suggests that rural diets are especially dynamic and that fruit consumption tends to decline during the dry season.[21,25] This dynamic is particularly pronounced in Tanzania, where approximately 80% of rural households primarily depend on home-grown FAV yields.[26,27]

Nutritional outcomes and FAV consumption are also shaped by a range of socio-demographic factors, including education, income, marital status, household size, and gender.[9,22,28,29] Diet and health are closely related, as adequate nutrition supports immune function and the management of chronic diseases, including HIV and hypertension.[30,31] This further stresses the importance of stable access to nutrient-rich foods, like fruits and vegetables, in the Tanzanian health context. Despite recognition that seasonality and other socio-demographic determinants influence dietary diversity and intake, few studies have empirically examined the stability of FAV intake across seasons in Northwestern Tanzania.[32,33]

In Northwestern Tanzania, household food access is shaped by local environmental conditions, making the area particularly relevant for examining dietary stability. The Kisesa ward in the Magu District of Northwestern Tanzania is largely rural, with most residents engaged in subsistence farming or small-scale trade.[34,35] The agricultural sector is particularly sensitive to climatic variability, as irregular rainfall and extended dry spells may undermine crop production and contribute to food insecurity.[36]

Rainfall in Northwestern Tanzania follows a bimodal pattern, with the Vuli (short rains) from October to December and the Masika (long rains) from March to May.[37] Most households cultivate staple crops, including maize, paddy, sorghum, cassava, sweet potatoes, and pulses.[35] Almost all households in Northwestern Tanzania grow food crops, but poorer households' harvests last only a few months, after which they rely on income from other economic activities to meet dietary needs.[38] Limited market access and transportation infrastructure in rural Tanzanian areas, like the Magu District, further restrict access to fruits and vegetables; as a result, fruit and vegetable intake in this setting is highly sensitive to seasonal and climatic fluctuations throughout the year.[39]

Although national surveys such as STEPS provide population-level estimates of FAV intake, they rely on cross-sectional assessments and do not capture seasonal variation at the individual level. Existing Tanzanian studies have examined dietary diversity, food security, or market dynamics rather than longitudinal intake patterns. There remains limited empirical evidence on how FAV intake fluctuates seasonally and which socio-demographic groups are most vulnerable to instability. This study aimed to describe FAV intake and its stability across wet and dry seasons in rural and semi-urban areas of Northwestern Tanzania and identify associated socio-demographic and health factors. Although this approach does not capture real-time intra-individual seasonal variation, it provides population-level insights into perceived seasonal differences in dietary intake, which are relevant in settings where longitudinal dietary data are unavailable.

2. Methodology

2.1 Study design and setting

The Magu Health and Demographic Surveillance System (Magu HDSS) was established in 1994 in a ward called Kisesa, Magu District, Mwanza Region, Northwestern Tanzania, as an open community cohort to study HIV epidemiology. Regular serosurveys have been conducted within the Magu HDSS, targeting all eligible individuals aged 15 years and older. Between 1994 and 2016, eight epidemiological surveys were carried out at roughly three-year intervals.[40]

During each survey round, a temporary clinic was established in each of seven villages. At these clinics, interviews were conducted to collect information on socio-demographic characteristics, birth history, sexual behavior, knowledge and attitudes towards HIV, experience with HIV counseling and treatment (HCT), and screening for other health issues.[41] Serosurvey data were collected electronically and linked to the other HDSS data through a unique household identifying number.

This cross-sectional secondary analysis used data from the seventh round of the Serosurveys, conducted in 2012/2013, which included 7,620 participants. Although the data were collected over a decade ago, they remain relevant because structural determinants of diet in Northwestern Tanzania, such as agricultural dependence and seasonal rainfall patterns, have remained largely consistent. Furthermore, more recent national surveys continue to report a high prevalence of inadequate FAV intake, suggesting that insufficient consumption persists as a public health concern.[18]

As this study represents a secondary analysis of the Sero 7 survey, which was primarily designed to monitor HIV epidemiology, only variables relevant to the current study objectives were extracted from the larger dataset, including FAV intake measures, socio-demographic characteristics, economic activity, HIV status and HCT uptake, self-reported diabetes and hypertension, and clinician-measured anthropometrics (height and weight). Other variables available in Sero 7 (e.g., sexual behavior, reproductive history, HIV knowledge) were not included because they were not pertinent to the research questions of this analysis.

Any participant aged 15+ who completed the Sero 7 Survey was included. Participants were excluded if they had missing data, showed discrepancies in their responses (e.g., indicating 'yes' to having received formal education but reporting zero years of schooling), or answered 'don't know' to any question. All excluded participants were recorded and counted. The final analytical sample, including participants with and without HCT data, was 7,226 individuals.

2.2 Measures

2.2.1 Fruit and vegetable intake

FAV intake was measured using participant responses to the food section of the questionnaire. Illustrated cards were used to show common fruits and vegetables and their average portion sizes; these cards helped participants recall their intake and reduce errors in estimating standard portion sizes. In the Tanzanian context, fruits and vegetables are often consumed as part of mixed dishes (e.g., stews and sauces), which may complicate accurate estimation of portion sizes. Although illustrated portion cards were used to aid recall, some degree of misclassification or measurement error in FAV intake is likely.

Participants were asked whether they consumed fruits or vegetables during the wet and dry seasons, the frequency of consumption in a typical week during that season, and the number of portions typically consumed on days when fruits or vegetables were eaten. The average servings per day of combined fruit and vegetables in the dry versus wet season were the main outcome variables. These measures were calculated using the equation $\left[\frac{(\frac{days\ consumed\ FAV}{week}) \times (\frac{portions\ of\ FAV}{day})}{7}\right]$. Previous studies have also relied on self-reported dietary assessment methods, including food-frequency based approaches, which supports the comparability of findings.[18]

Participants who consumed <5 servings of fruits and/or vegetables per day were categorized as having "inadequate intake," and those who consumed ≥5 servings per day were categorized as having "adequate intake" during either season. This classification is based on the WHO recommendation of ≥5 servings per day and has previously been applied in Tanzania.[9]

To capture the seasonal stability of FAV intake, a four-category FAV stability variable was constructed:

1. Stable adequate: consumed ≥5 servings per day in both wet and dry seasons
2. Wet intake: consumed ≥5 servings per day in the wet season but <5 servings per day in the dry season
3. Dry intake: consumed <5 servings per day in the wet season but ≥5 servings per day in the dry season
4. Stable inadequate: consumed <5 servings per day in both wet and dry seasons

This variable reflects the adequacy and seasonal stability of FAV intake, enabling the

identification of individuals who maintain sufficient intake year-round versus those vulnerable to seasonal declines. The wet and dry intake categories are particularly relevant in policy and health programming as they specify the seasonality of inadequate intake, as opposed to a measure of non-specified directional change.

Because the Sero 7 questionnaire was not designed as a full dietary assessment tool, only a restricted set of diet-related variables was available. Key food groups typically required to analyze complete dietary patterns, as in other Tanzanian studies, were not collected; therefore, this analysis focuses solely on seasonal variation in FAV intake.[21,25]

2.2.2 Socio-demographic characteristics

Participants were interviewed to obtain sociodemographic and health-related characteristics, including age (years), sex (male/female), height (cm), weight (kg), village of residence, educational attainment, marital status, HIV status, use of health services, self-reported presence of diabetes and hypertension, and participation in income-generating activities.

Villages were categorized as semi-urban (Kanyama, Kisesa) or rural (Igekemaja, Kitumba, Isangijo, Ihayabuyaga, Welamasonga). Age was grouped into 15–24, 25–49, and 50+ years. Marital status was categorized as never married, married (monogamous or polygamous), or divorced/ widowed/separated. Body mass index (BMI) was calculated using the formula [weight (kg) / (height (m))^2] and categorized as underweight (<18.5), normal (18.5–24.9), or overweight/obese (≥25.0), according to WHO guidelines.[42] Educational status was categorized as no formal education, primary education (completion of primary schooling), or secondary education (secondary school, college, or university). Participants were asked whether they engaged in any income-generating activity (Yes/No). For those reporting participation, the primary income-generating activity was categorized as farming, skilled labor (e.g., driving, professional, skilled labor), or unskilled labor/other (e.g., business, unskilled labor, other).

2.2.3 Health characteristics

HIV status was determined based on clinic HIV test results (positive/negative). HCT uptake was measured using responses to the question: "How many times have you had HIV counseling and testing?", in either a voluntary or provider-initiated context. Participants reporting zero times were categorized as having no HCT uptake, while those reporting one or more tests were categorized as having HCT uptake. HCT uptake was analyzed as a binary variable (any previous HCT/none). This operationalization aligns with approaches used in previous studies examining HIV testing behaviors using binary outcomes.[43,44]

Self-reported diabetes and hypertension status were measured using the response to the questions, "Have you ever been told by a doctor or other health worker that you have raised blood sugar or diabetes?" and "Have you ever been told by a doctor or other health worker that you have high blood pressure or hypertension?", respectively. Both variables were coded as binary outcomes (Yes/No). Although clinical measurements were not collected in Sero 7, self-reported diabetes and hypertension have been used as proxy indicators in similar population-based studies.[45] Diabetes and hypertension were self-reported and not clinically verified, which may result in underreporting and misclassification; this limitation is considered in interpretation of results.

2.3 Statistical analysis

Descriptive statistics were calculated to summarize participant sociodemographic and health characteristics in the 7,226 individual sample. Continuous variables were reported with median

and IQR, while categorical variables were presented with counts and percentages. Continuous FAV intake (servings/day) between the wet and dry seasons was compared using a paired t-test for normally distributed data, with non-parametric Wilcoxon signed-rank tests conducted as a non-parametric sensitivity analysis. Changes in adequacy of intake (<5 vs. ≥5 servings/day) between seasons were assessed using McNemar's test. Associations between dietary stability and socio-demographic factors were examined using multinomial logistic regression. Bivariate models were first fitted for each predictor separately to estimate relative risk ratios (RRR) and 95% confidence intervals. All variables showing marginally significant associations ($p<0.10$) in bivariate analysis were included in the multivariable model to obtain adjusted RRRs. Stable adequate served as the reference category for all models. When expected cell counts were below 5 in certain subgroups, indicating potential data sparsity and risk of unstable model estimates, the categories were collapsed into two groups: stable intake (stable adequate + stable inadequate) and changed intake (wet + dry); these binary analyses focused on whether participants' intake changed across seasons rather than on season-specific adequacy. This collapse required analysis using binary logistic regression and limited the ability to distinguish between improvements and declines in adequacy across seasons.

Complete-case analysis was used for all variables with low missingness (5%). Because HCT had substantial missingness (16.3%), it was excluded from 2 of the multivariable models to prevent major reductions in sample size. Instead, HCT was examined separately using descriptive and bivariate analyses and an additional limited multivariable model. These findings were interpreted cautiously, with no imputation methods being applied. Figure 1 provides a summary of participant inclusion, including missing data. Associations were considered significant at $p < 0.05$. All analyses were conducted using STATA/BE 19.5F and RStudio (version 2023.12.1+402).

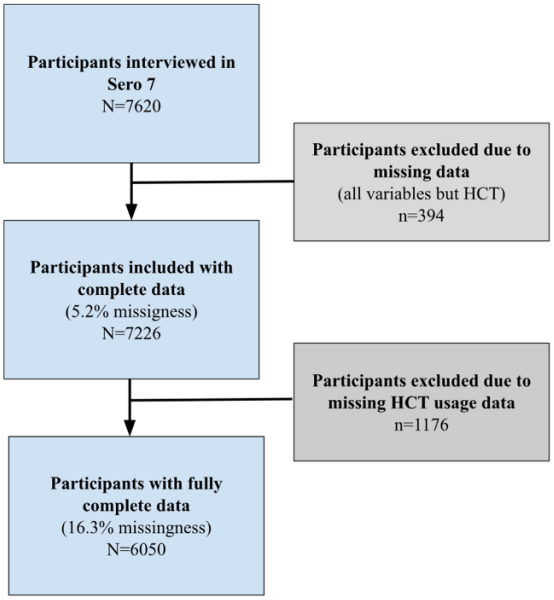

Figure 1. Participant inclusion flow chart.

3. Results

3.1 Population description

Table 1 provides a detailed summary of the sociodemographic, health, and dietary characteristics of the study population. The final analytical sample, including participants with and without HCT data, was comprised of 7,226 individuals, the majority of whom were female (62.65%). Participants were predominantly between 25 and 49 years of age (41.63%), with smaller proportions in the 15–24 (37.14%) and 50+ (21.23%) age groups. Most respondents resided in rural areas (66.04%), while the remaining third lived in semi-urban settings.

With respect to nutritional status, most participants had a normal BMI (69.62%), whereas 20.19% were underweight and 10.19% were overweight or obese. More than half of the participants were married (52.89%), while only 14.99% were widowed, divorced, or separated, and

one-third had never been married. The majority identified as ethnically Sukuma (94.33%). Two-thirds of the population (64.81%) reported participating in an income-generating activity, most frequently farming (78.05%); smaller proportions reported skilled (4.53%) and unskilled labor/other activities (17.42%) (Table 1).

HIV prevalence in the sample was 6.95%. Self-reported diabetes and hypertension were uncommon, reported by 0.43% and 2.68% of participants, respectively (Table 1). Of the 6,050 respondents with responses for the HIV counseling and testing question, uptake was 43.02%.

The high proportion of rural residents and farming households is particularly relevant given the study's focus on seasonal dietary stability, as these groups are more directly impacted by agricultural cycles and rainfall variability.

3.2 Patterns of fruit and vegetable consumption

Overall, fruit and vegetable consumption was low among participants. Median combined FAV intake was 1.71 servings per day (IQR: 1.29–2.36) (Table 1). Intake varied by season, with higher consumption during the wet season. Median daily FAV intake during the wet season was 2.29 servings (IQR: 1.71–3.29), compared with 1.00 servings in the dry season (IQR: 0.57–1.71).

When fruit and vegetable intake were examined separately, participants reported slightly higher fruit consumption than vegetable consumption in both seasons. Median daily fruit intake was 0.93 servings (IQR: 0.57–1.36), while median vegetable intake was 0.79 servings per day (IQR: 0.50–1.14). Both fruits and vegetables were consumed a median of 3.50 days/week throughout the year.

Figure 2 presents mean daily FAV intake in the wet and dry seasons across sociodemographic, socioeconomic, and health-related subgroups. Although the magnitude of difference varied by subgroup, the consistent pattern indicates higher FAV consumption during the wet season across the entire study population.

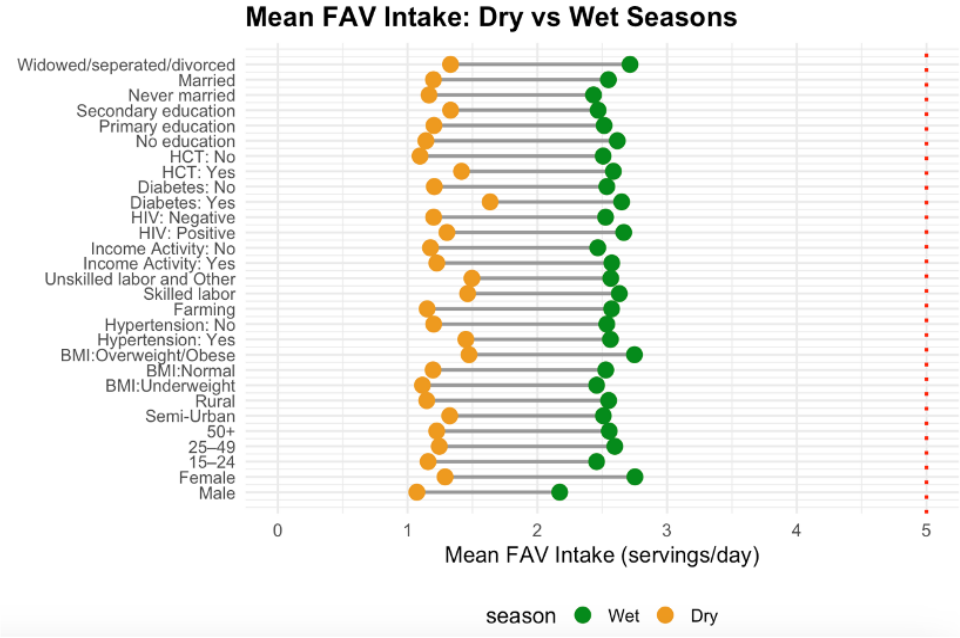

Figure 2. Mean FAV intake for wet and dry seasons by sociodemographic and health characteristics.

3.3 Seasonal differences in fruit and vegetable intake

Table 2 presents the cross-tabulation of FAV intake adequacy across the wet and dry seasons. Overall, only a small proportion of participants met adequate FAV intake in either season. During the wet season, 261 participants (3.61%) achieved adequate FAV intake, while 6,965 (96.39%) did not. In the dry season, adequacy further declined: only 42 participants (0.58%) met adequate intake, while 7,184 (99.42%) were inadequate.

Across seasons, only 23 participants (0.32%) achieved adequate intake in both the wet and dry seasons. An additional 238 (3.29%) had adequate intake in the wet season but not the dry season, while 19 (0.29%) improved from inadequate in the wet season to adequate in the dry season. The majority, 6,946 participants (96.12%), reported inadequate intake in both seasons.

There was a statistically significant difference in FAV intake between the wet and dry seasons. On average, participants consumed substantially more FAV during the wet season, with a mean increase of 1.33 servings per day (95% CI: 1.30–1.35; $t = 68.41$, $p < 0.01$). The Wilcoxon signed-rank test confirmed this difference ($Z = 68.58$; $p < 0.01$), indicating a consistent seasonal shift in intake.

The odds of achieving adequate intake were also markedly higher in the wet season. Participants had 12.53 times higher odds of meeting recommended intake levels in the wet season compared with the dry season (95% CI: 7.85-21.18; $\chi^2 = 186.62$; $p < 0.01$). Detailed results are presented in Table 3.

3.4 Bivariate regression

Table 4 summarizes the bivariate logistic regression results comparing three FAV stability categories (Wet Intake, Dry Intake, and Stable Inadequate) relative to the reference group (Stable Adequate). These findings should be interpreted cautiously due to small cell counts for some variables and resulting wide confidence intervals.

Females had a significantly lower relative risk of Stable Inadequate intake (RRR = 0.07; 95% CI: 0.01–0.55; p = 0.01) compared with males. Rural residence was associated with a higher relative risk across all three FAV stability categories compared with semi-urban residents: Wet Intake (RRR = 10.52; 95% CI: 4.16–26.62; $p < 0.01$), Dry Intake (RRR = 4.06; 95% CI: 1.11–14.81; p= 0.03), and Stable Inadequate (RRR = 3.56; 95% CI: 1.51-8.40; $p<0.01$). Overweight or obese participants had a significantly lower risk of Wet Intake (RRR = 0.27; 95% CI: 0.14–0.71; $p < 0.01$) and Stable Inadequate intake (RRR = 0.23; 95% CI: 0.10-0.57; $p < 0.01$) relative to those with normal BMI.

Of those economically-active participants, those who worked Unskilled/other labor had a lower relative risk of Wet Intake compared to those who farmed as their main activity (RRR = 0.42; 95% CI: 0.08–0.57; $p<0.01$).

Of those limited participants with responses for HCT usage, uptake was associated with lower relative risk. Individuals who had ever used HCT services were significantly less likely to experience Wet Intake (RRR = 0.26; 95% CI: 0.09–0.73; p = 0.01) and less likely have Stable Inadequate intake (RRR = 0.22; 95% CI: 0.08–0.60; $p < 0.01$), compared with those who had never used HCT services.

No other sociodemographic, economic, or health variables were significantly associated with FAV stability categories, but the 50+ age group and income-generating participation were both marginally associated with Dry Intake and Stable Inadequate, respectively. Due to low cell counts, self-reported diabetes and education results were unstable; a corresponding binary analysis for both variables is presented in the Appendix, with no statistically significant findings.

3.5 Multivariable regression

Table 5 presents the adjusted multinomial logistic regression results comparing the three FAV stability categories (Wet Intake, Dry Intake, and Stable Inadequate) with the reference group, Stable Adequate intake. Analyses were conducted in three samples based on data availability. Results for the full analytic sample are presented first. Of the 7,620 participants in the Sero 7 survey, 7,226 were included in this analysis after excluding those with missing values on key variables. This sample represents the broadest available dataset for examining FAV stability categories and serves as the primary reference for comparisons with the two subsamples presented subsequently.

3.5.1 Full analytic sample (N=7,226)

Females had a lower relative risk of Stable Inadequate intake compared to males (RRR = 0.10; 95% CI: 0.01–0.72; p=0.02). Those aged 50 years or older had a significantly higher relative risk of Wet Intake (RRR = 10.86; 95% CI: 1.26–93.69; p=0.03) and Dry Intake (RRR = 15.41; 95% CI: 1.33–178.61; p=0.03), compared to those aged 15-24. Compared with semi-urban residents, rural participants had a significantly higher relative risk of experiencing Wet Intake (RRR = 10.86; 95% CI: 3.85–26.62; p<0.01) and Stable Inadequate intake (RRR = 2.98; 95% CI: 1.21–7.30; p=0.02). Overweight or obese participants had a 63% lower relative risk of Stable Inadequate intake (RRR = 0.37; 95% CI: 0.15–0.93; p = 0.04) compared with those with normal BMI. Income-generating activity participation showed no statistically significant association with any FAV stability category after adjustment.

3.5.2 Subsample of economically active participants (N= 4,683)

Among economically active participants, only resident area and BMI factors had significant associations. Rural residence had a higher relative risk of Wet Intake (RRR = 9.99; 95% CI: 3.11-32.10; p<0.01) and Dry Intake (RRR = 6.47; 95% CI: 1.11-37.65; p=0.04), compared to semi-urban participants. Overweight or obese participants had a significantly lower risk of Stable Inadequate intake (RRR = 0.36; 95% CI: 0.13–0.95; p=0.04) relative to those with normal BMI.

3.5.3 Subsample of participants with HIV counselling and testing usage data (N= 6,050)

Of those participants with a response to HCT usage, females had a lower relative risk of Stable Inadequate intake compared to males (RRR = 0.10, 95% CI: 0.01–0.76; p=0.03). In line with the other models' findings, rural residents had a higher relative risk of Wet Intake (RRR = 7.36; 95% CI: 2.63-20.63; p<0.01), compared to semi-urban participants. HCT uptake had a lower relative risk of Stable Inadequate intake (RRR = 0.37, 95% CI: 0.13–1.07), although this association was insignificant (p = 0.07). No other independent variable showed statistically significant associations with any FAV intake category.

4. Discussion

Overall, FAV intake in this rural/semi-urban Tanzanian population was low, with a median intake of 1.71 servings/day. Only 3.56% of individuals achieved adequate intake in at least one season, with less than 1% meeting recommended intake in both seasons. FAV intake showed strong seasonal variation, with significantly higher intake in the wet season compared with the dry season. Wet-season intake exceeded dry-season intake by 1.33 servings per day on average (95% CI: 1.30–1.45), with a 12.53-fold increase in the odds of adequate consumption.

Multivariable analyses identified several significant associations, although some estimates were imprecise due to wide confidence intervals and small cell counts. In adjusted models, female sex and

being overweight/obese were associated with a lower risk of inadequate intake, whereas rural residence and being aged 50+ were associated with a higher risk of inadequate or seasonally unstable intake. Associations with income-generating activity and HCT uptake attenuated after adjustment.

The low overall intake observed aligns with national findings from the Tanzania STEPS surveys, which similarly report average daily FAV intake of 1.5–2.5 servings.[18,19] Comparable patterns of inadequate consumption have been documented across SSA studies and STEP surveys.[16,17,46,47] This pattern likely reflects broader structural barriers such as limited storage capacity, low purchasing power, competing food priorities, limited nutritional knowledge, as well as shifting diets.[48,49] Data from high-income settings also supports low-intake, illustrating the scoping inadequacies of diets worldwide.[50]

Seasonal variation in FAV availability and intake is also well-established, with dry/post-harvest periods consistently associated with decreased dietary diversity and reduced access to fresh produce.[23,25,48,51] Considering the high prevalence of farming as an income-generating activity, FAV intake is likely to be influenced by seasonality, as agricultural cycles affect food availability, access, utilization, and supply stability in Tanzania.[25] Diets are often more diverse in the rainy season, especially with respect to intake of FAV, such as vitamin A-rich dark leafy greens and fruits.[51] This further highlights the nutritional impact of fluctuating diets, as individuals' intakes of beneficial nutrients vary throughout the year, reducing the optimization of FAV benefits.

In line with this study, Msambichaka et al. (2018) found that women had a lower risk for inadequate intake.[9] While this pattern was not statistically significant across all literature, a pattern of higher FAV intake among women is consistently observed in STEPS findings.[18,19] This finding could be a result of women's central role in preparing and managing food resources in Tanzanian households, as well as greater reported awareness of healthy diets and seeking nutritional guidance.[51] Furthermore, men consume their meals out of the home more often than women, which may impact their access and consumption of FAV.[52,53]

The observed associations between rural residence and FAV stability differ in some literature, suggesting that rural households may have greater access to FAV due to proximity to agricultural production; however, they are consistent with studies that incorporate seasonal analyses, which more effectively capture dietary variation.[7,48] Rural regions often face shortages post-harvest, limited storage capacity, reduced market access, and lower financial resources to buffer seasonal price increases.[18,21,25] Affordability is a well-documented barrier to the intake of adequate diets for rural households.[33,51,54] Furthermore, to meet the recommended FAV intake, the increase in food expenditure would be more significant in rural areas than urban ones.[49] Notably, rural residence remained a significant predictor across adjusted models, strengthening confidence in this association.

The observed inadequate intake among adults aged 50 years or older is supported by evidence from SSA showing that most elderly adults have limited dietary adequacy.[55,56] FAV intake decreases with age, although some Tanzanian findings suggest the opposite, indicating possible regional or cohort differences.[9,48,55] Older adults may face barriers to adequate FAV intake due to reduced income, physical limitations affecting food production, reliance on others for food procurement, and prioritization of staple foods over fruits and vegetables.[57] In rural settings, older adults may be disproportionately affected by seasonal food shortages due to limited mobility and resources. Furthermore, few interventions have focused on the nutritional status of this population, since women of

reproductive age, infants, and children are often prioritized due to their specific needs.[55,56]

Groups identified with a higher risk of inadequate FAV intake in this study, including rural residents, older adults, and men, are also shown to have a higher prevalence of hypertension in Tanzania.[58] As prevalence for related NCDs is rising, this link suggests that seasonal dietary inadequacy may contribute to existing NCD vulnerabilities.[1]

Limited literature exists on BMI and FAV, making direct comparisons difficult; however broader dietary diversity and food insecurity research highlights various pathways. Korir et al. (2024) found a positive association between dietary diversity and BMI, suggesting that more varied food intake may be linked to overweight/obese BMI categories.[59] While FAV has a positive impact on an individual's diet, its association to higher BMI may be diminished by unhealthy eating patterns like high-calorie or high-fat diets. Dietary diversity does not necessarily equate to nutritional adequacy, so this relationship should be considered critically and further studied. Food security may also be a mediating factor, as food-secure women were found to have higher median BMI values.[27,60] Seasonal fluctuations in FAV availability may have a smaller impact among food-secure individuals, which could support the lower risk of inadequate intake observed among overweight/obese participants.[27]

The positive, though not statistically significant, association between HCT uptake and adequate intake is consistent with Msambichaka et al. (2018) linking health-seeking behaviors to higher FAV intake, although evidence remains preliminary.[9] Another study identified that increased preventative health care usage was associated with healthy behaviors; even though FAV intake was not significantly associated, it supports a potential pathway for understanding the direction of association.[61] This positive but non-significant association may represent a preliminary pathway, but requires further exploration.

4.1 Strengths and limitations

This is among the few studies in Tanzania that focus specifically on FAV intake. The Sero 7 dataset offered a large sample size, which was important given the low incidence of adequate intake, and ensured socio-demographic attributes were represented in the sample. Considering the changing nutritional and epidemiological landscape of Tanzania, this study supports previously identified patterns of FAV intake and highlights a need for additional, more current research.

This study was based on a secondary analysis of cross-sectional data, which limits the ability to infer causal relationships between FAV stability and health or socio-demographic factors. The data were collected in 2012–2013; thus, findings may not fully reflect recent dietary patterns. In addition, self-reported information on FAV intake and disease diagnoses for hypertension and diabetes may be subject to recall bias. The use of a single dietary indicator may not capture the full complexity of dietary diversity, total nutrient intake, or intra-seasonal fluctuations in intake. Furthermore, the Sero 7 questionnaire did not distinguish between staple crops and other vegetables. No imputation methods were used; complete-case analysis was applied because missingness was minimal for most variables. Despite low missingness, excluding participants with incomplete responses may have introduced selection bias if missingness was not random. Individuals with lower literacy or education may be more likely to provide incomplete responses and may also be more vulnerable to inadequate FAV intake, potentially leading to underestimation of the true magnitude of dietary inadequacy. Missingness in the HCT variable was higher and was addressed analytically by separating HCT in the analysis. Furthermore, no information about other potential modifiers, such as an awareness of the components

of a healthy diet, purchasing power, and accessibility, was collected. Finally, because the data come from one geographic region in Northwestern Tanzania, generalizability to other populations is limited.

4.2 Recommendations

This study provides evidence of significant seasonality in FAV intake in Northwestern Tanzania and associated determinants. These findings highlight the need for season-conscious interventions that stabilize year-round access and consumption of FAV. Future studies should consider more frequent and longitudinal dietary assessments, such as those employed in initiatives like the CGIAR-led Fruit and Vegetables for Sustainable Healthy Diets Initiative, which utilize repeated measurements to better capture seasonal dietary variation.

Research exploring the specific cultural, environmental, and economic mechanisms that shape intake is essential to illuminating why such associations exist. Given the epidemiological transition in Tanzania, studies should also explore seasonal dietary inadequacy as a specific risk factor for NCDs. Furthermore, evaluative studies on interventions like community gardens, improved storage and processing infrastructure, and subsidies for FAV during the dry season would provide evidence for practice.

While current national action plans acknowledge the importance of improving FAV intake, such reporting lacks a seasonal dimension. Future policies should emphasize education on the importance of FAV intake and strengthening the food system to reduce potential barriers like affordability. Studies have shown that improving knowledge through education is not enough to improve diets in Tanzania and that structural interventions are also necessary.[48] Furthermore, specific populations, like rural residents, males, and older adults, who were associated with inadequate intake, should be targeted as well.

5. Conclusion

This study confirms low FAV intake in this rural and semi-urban Tanzanian population and demonstrates an association between seasonal inadequacy with socio-demographic and health factors. Adequate intake was uncommon year-round and even rarer in the dry season. The significant seasonal fluctuation highlights the role of environmental conditions and socio-demographic determinants in shaping dietary patterns. Rural residence and older age were associated with a higher risk of inadequate consumption in both individual seasons and over the combined period. Women and individuals who were overweight/obese had a lower risk for inadequate intake year-round. These findings emphasize the need for additional research on dietary patterns, with a focus on FAV and seasonality. Future interventions should emphasize stability, affordability, and accessibility of FAV throughout the year. Tailored strategies for higher-risk populations are particularly important. As Tanzania's population and nutritional landscapes change, addressing FAV fluctuations and adequacy is consequential.

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Table 1. Sociodemographic, health, and dietary characteristics of study participants

Variable	Groups	N=7226	Females	Males
		n (%)	n (%)	n (%)
All			4527 (62.65)	2699 (37.35)
Age Group	15-24	2684 (37.14)	1453 (54.14)	1231 (45.86)
	25-49	3008 (41.63)	2087 (69.38)	921 (30.62)
	50+	1534 (21.23)	987 (64.34)	547 (36.66)
Resident Area	Semi-Urban	2454 (33.96)	1683 (68.58)	771 (31.42)
	Rural	4772 (66.04)	2844 (59.60)	1928 (40.40)
BMI	Underweight	1459 (20.19)	760 (52.09)	699 (47.91)
	Normal	5031 (69.62)	3164 (62.89)	1867 (37.11)
	Overweight/Obese	736 (10.19)	603 (81.93)	133 (18.07)
Marital Status	Never married	2321 (32.12)	1003 (43.21)	1218 (56.79)
	Married	3822 (52.89)	2550 (66.72)	1272 (33.28)
	Widowed/Separated/Divorced	1083 (14.99)	974 (89.94)	109 (10.06)
Ethnicity	Sukuma	6816 (94.33)	4244 (62.27)	2572 (37.73)
	Other	410 (5.67)	283 (69.02)	127 (30.98)
Income-Generating	Yes	4683 (64.81)	2943 (62.84)	1740 (37.16)
	No	2543 (35.19)	1584 (62.29)	959 (37.71)
Economic activity n= 4683	Farming	3655 (78.05)	2277 (62.30)	1378 (37.70)
	Skilled labor	212 (4.53)	93 (43.87)	119 (56.13)
	Unskilled labor/other	816 (17.42)	573 (70.22)	243 (29.78)
HCT uptake n= 6050	Yes	2603 (43.02)	1614 (62.01)	989 (37.99)
	No	3447 (56.98)	2035 (59.04)	1412 (40.96)
HIV Status	Positive	502 (6.95)	357 (71.12)	145 (28.88)
	Negative	6724 (93.05)	4170 (62.02)	1554 (37.98)
Diabetes	Yes	31 (0.43)	18 (58.06)	13 (41.94)
	No	7195 (99.57)	4509 (62.67)	2686 (37.33)

Hypertension	Yes	194 (2.68)	166 (85.57)	28 (14.43)
	No	7032 (97.32)	4361 (62.02)	2671 (37.98)
Education	No education	2059 (28.49)	1616 (78.48)	443 (21.52)
	Primary	3912 (54.14)	2380 (60.84)	1532 (39.16)
	Secondary	1255 (17.37)	532 (42.31)	724 (57.69)
	Median	**Interquartile Range**		
Age	31	20-46		
BMI	20.71	18.92-22.68		
FAV intake combined seasons	1.71	1.29-2.36		
FAV intake wet season	2.29	1.71-3.29		
FAV intake dry season	1.00	0.57-1.71		
Fruit intake combined seasons	0.93	0.57-1.36		
Vegetable intake combined seasons	0.79	0.50-1.14		
Average days per week fruit	3.50	2.50-4.50		
Average days per week vegetables	3.50	2.50-4.50		

Intake measured in servings/day.

Table 2. Adequacy of FAV intake by season

	Dry Season		
Wet Season	Adequate	Not Adequate	**Total**
Adequate	23	238	261
Not Adequate	19	6946	6965
Total	42	7184	7226

Table 3. Comparison of FAV intake between wet and dry seasons - continuous and binary analyses

Outcome type	Test	Measure	Estimate (Wet-Dry)	95% CI	Test Statistic	P-value
Continuous Outcome (servings/day)	Paired t-test	Mean difference	1.33	(1.30, 1.35)*	68.41	<0.01
	Wilcoxon Signed Rank				68.58	<0.01
Binary Outcome (Adequate vs. Inadequate intake)	McNemar's Chi Square	OR	12.53	(7.85, 21.18) *	186.62	<0.01

Table 4. Bivariate multinomial logistic regression results for FAV stability categories

		Wet Intake		Dry Intake		Stable Inadequate	
Variable		**RRR (95% CI)**	**p-value**	**RRR (95% CI)**	**p-value**	**RRR (95% CI)**	**p-value**
Sex	Male	(ref)	-	(ref)	-	(ref)	-
	Female	0.18 (0.02, 1.37)	0.10	0.10 (0.01, 0.91)	0.04	0.07 (0.01, 0.55)*	0.01
Age Group	15-24	(ref)	-	(ref)	-	(ref)	-
	25-49	0.98 (0.38, 2.53)	0.97	0.75 (0.18, 3.13)	0.69	0.51 (0.21, 1.25)	0.14
	50+	5.62 (0.67, 47.26)	0.11	8.40 (0.76, 93.34)	0.08	3.96 (0.49, 32.24)	0.20
Resident Area	Semi-Urban	(ref)	-	(ref)	-	(ref)	-
	Rural	10.52 (4.16, 26.62)*	<0.01	4.06 (1.11, 14.81) *	0.03	3.56 (1.51, 8.40)*	<0.01
BMI	Normal	(ref)	-	(ref)	-	(ref)	-
	Underweight	1.58 (0.34, 7.26)	0.56	0.93 (0.11, 7.59)	0.95	1.90 (0.43, 8.43)	0.40
	Overweight/Obese	0.27 (0.10, 0.71)*	<0.01	0.35 (0.08, 1.60)	0.18	0.23 (0.10, 0.57)*	<0.01
Income-Generating	Yes	(ref)	-	(ref)	-	(ref)	-
	No	1.78 (0.59, 5.44)	0.31	2.19 (0.51, 9.33)	0.29	2.62 (0.89, 7.70)	0.08
Economic activity n=4683	Farming	(ref)	-	(ref)	-	(ref)	-
	Skilled labor	0.67 (0.08, 5.80)	0.71	1.13 (0.06, 21.09)	0.93	0.51 (0.06, 4.08)	0.53
	Unskilled labor/Other	0.21 (0.08, 0.57)*	<0.01	0.50 (0.11, 2.27)	0.37	0.22 (0.09, 0.56)*	<0.01
HCT uptake n=6050	No	(ref)	-	(ref)	-	(ref)	-
	Yes	0.26 (0.09, 0.73)*	0.01	0.37 (0.08, 1.39)	0.13	0.22 (0.08, 0.60)*	<0.01

HIV Status	Negative	(ref)	-	(ref)	-	(ref)	-
	Positive	1.00 (0.28, 3.56)	0.99	1.25 (0.22, 7.05)	0.80	0.48 (0.14, 1.62)	0.24
Diabetes	Yes						
	No			***			
Hypertension	No	(ref)	-	(ref)	-	(ref)	-
	Yes	0.96 (0.12, 7.89)	0.97	1.22 (0.07, 20.94)	0.89	0.59 (0.8, 4.42)	0.61
Education	No education						
	Primary						
	Secondary			***			
Marital Status	Never married	(ref)	-	(ref)	-	(ref)	-
	Married	1.30 (0.49, 3.46)	0.60	1.40 (0.35, 5.67)	0.64	0.95 (0.37, 2.42)	0.92
	Widowed/Separated/Divorced	1.54 (0.43, 5.56)	0.51	0.70 (0.09, 5.53)	0.73	0.80 (0.23, 2.73)	0.72

ref= Reference category: Stable Adequate. Results presented as Relative Risk Ratios (RRR) with 95% Confidence Intervals. RRR values >1 indicate increased relative risk of being in the comparison category vs. Stable Adequate; RRR <1 indicates decreased risk. *Indicates $p < 0.05$. ***Indicates model instability due to low cell counts; corresponding binary analysis presented in Appendix.

Table 5. Adjusted multinomial logistic regression results for FAV stability categories

Variable		Wet Intake		Dry Intake		Stable Inadequate	
		RRR (95% CI)	p-value	RRR (95% CI)	p-value	RRR (95% CI)	p-value
A. Full participant population (N = 7,226)							
Sex	Male	(ref)	-	(ref)	-	(ref)	-
	Female	0.24 (0.03, 1.82)	0.17	0.12 (0.01, 1.10)	0.06	0.10 (0.01, 0.72)*	0.02
Age Group	15-24	(ref)	-	(ref)	-	(ref)	-
	25-49	2.14 (0.76, 6.09)	0.15	1.58 (0.33, 7.67)	0.57	1.21 (0.45, 3.25)	0.7
	50+	10.86 (1.26, 93.69)*	0.03	15.41 (1.33, 178.61)*	0.03	7.68 (0.92, 64.03)	0.06
Resident Area	Semi-Urban	(ref)	-	(ref)	-	(ref)	-
	Rural	10.13 (3.85, 26.62)*	<0.01	3.93 (1.02, 15.07)	0.05	2.98 (1.21, 7.30)*	0.02
BMI	Normal	(ref)	-	(ref)	-	(ref)	-
	Underweight	1.29 (0.27, 6.04)	0.75	0.65 (0.08, 5.45)	0.69	1.29 (0.29, 5.81)	0.74
	Overweight/Obese	0.47 (0.17, 1.28)	0.14	0.53 (0.10, 2.59)	0.43	0.37 (0.15, 0.93)*	0.04
Income-generating	Yes	(ref)	-	(ref)	-	(ref)	-
	No	2.38 (0.70, 8.03)	0.16	2.71 (0.55, 13.23)	0.22	2.58 (0.80, 8.36)	0.11
B. Subsample: participants engaged in income-generating activities (N = 4,683)							
Sex	Male	(ref)	-	(ref)	-	(ref)	-
	Female	0.38 (0.05, 3.01)	0.36	0.13 (0.01, 1.36)	0.09	0.13 (0.02, 1.02)	0.05
Age Group	15-24	(ref)	-	(ref)	-	(ref)	-
	25-49	1.63 (0.43, 6.23)	0.48	1.34 (0.18, 10.06)	0.77	0.79 (0.22, 2.80)	0.71
	50+	5.71 (0.54, 60.24)	0.14	7.55 (0.40, 140.88)	0.18	3.82 (0.38, 37.87)	0.25

Resident Area	Semi-Urban	(ref)	-	(ref)	-	(ref)	-
	Rural	9.99 (3.11, 32.10)*	<0.01	6.47 (1.11, 37.65)*	0.04	2.56 (0.87, 7.52)	0.08
BMI	Normal	(ref)	-	(ref)	-	(ref)	-
	Underweight	1.72 (0.21, 14.19)	0.61	0.89 (0.05, 16.53)	0.94	1.59 (0.20, 12.49)	0.66
	Overweight/Obese	0.44 (0.15, 1.31)	0.14	0.45 (0.07, 2.93)	0.41	0.36 (0.13, 0.95)*	0.04
Economic activity	Farming	(ref)	-	(ref)	-	(ref)	-
	Skilled labor	1.58 (0.17, 14.52)	0.69	1.95 (0.10, 34.45)	0.66	0.68 (0.08, 5.70)	0.73
	Unskilled labor/ Other	0.59 (0.20, 1.78)	0.35	1.39 (0.27, 7.11)	0.69	0.47 (0.17, 1.30)	0.15
C. Subsample: participants with HCT usage responses (N = 6,050)							
Sex	Male	(ref)	-	(ref)	-	(ref)	-
	Female	0.24 (0.03, 1.91)	0.18	0.18 (0.02, 1.86)	0.15	0.10 (0.01, 0.76)*	0.03
Age Group	15-24	(ref)	-	(ref)	-	(ref)	-
	25-49	2.18 (0.75, 6.34)	0.15	1.66 (0.30, 9.11)	0.56	1.41 (0.52, 3.84)	0.50
	50+	8.85 (1.00, 78.04)	0.05	10.64 (0.81, 140.09)	0.07	6.14 (0.73, 51.76)	0.10
Resident Area	Semi-Urban	(ref)	-	(ref)	-	(ref)	-
	Rural	7.36 (2.63, 20.63)*	<0.01	2.76 (0.64, 11.90)	0.17	2.09 (0.80, 5.46)	0.13
BMI	Normal	(ref)	-	(ref)	-	(ref)	-
	Underweight	0.99 (0.21, 4.76)	0.99	0.94 (0.10, 8.16)	0.95	1.08 (0.24, 4.89)	0.93
	Overweight/Obese	0.47 (0.17, 1.32)	0.15	0.63 (0.122, 3.24)	0.58	0.38 (0.15, 0.98)	0.05
	Yes	(ref)	-	(ref)	-	(ref)	-

Income-generating	No	1.96 (0.56, 6.83)	0.29	1.77 (0.31, 10.23)	0.52	2.00 (0.61, 6.62)	0.25
HCT uptake	No	(ref)	-	(ref)	-	(ref)	-
	Yes	0.47 (0.15, 1.40)	0.17	0.54 (0.12, 2.45)	0.42	0.37 (0.13, 1.07)	0.07

ref=Reference category: Stable Adequate. Results presented as Relative Risk Ratios (RRR) with 95% Confidence Intervals. RRR values >1 indicate increased relative risk of being in the comparison category vs. Stable Adequate; RRR <1 indicates decreased risk. Models were adjusted for sex, residence area, BMI category, and marital status (and for income-generating activity and HCT uptake in the subsamples). *Indicates $p < 0.05$.

Table 6. Supplementary Binary Regression Analysis for Diabetes and Education

		Change/No Change	
Variable		**RR (95% CI)**	**p-value**
Diabetes	No	(ref)	-
	Yes	0.91 (0.13, 6.26)	0.92
Education	No education	(ref)	-
	Primary	0.79 (0.61, 1.04)	0.09
	Secondary	0.85 (0.59, 1.21)	0.37

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Seasonal Variation in Rock Hyrax Behavior and Energy Budget: A Comparative Study in Randilen Wildlife Management Area, Tanzania

Eric Feichthaler

Seasonal Variation in Rock Hyrax Behavior and Energy Budget: A Comparative Study in Randilen Wildlife Management Area, Tanzania

Eric Feichthaler[1]

[1] Department of Biology, Georgetown University, Washington, D.C., United States

Email: epf20@georgetown.edu
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Abstract

The rock hyrax (Procavia capensis) exhibits varying thermoregulation and foraging habits between seasons, which reflect the rock hyrax's overall behavior and energy budget. However, this relationship has not been sufficiently studied in Tanzania, as rock hyraxes are among the least studied mammals in East Africa. This observational study was conducted during a 10-day period in November 2025 on the kopje at Buti Main Gate in Randilen Wildlife Management Area (WMA) in Tanzania. The objectives were to analyze the social behavior and energy budget of rock hyraxes during the dry season at the gate of Randilen WMA and determine whether it differs from that of the wet season. Scan sampling was conducted twice daily (morning and evening) every 5 minutes to observe the behavior and activity state of all visible rock hyraxes. This rock hyrax population ranged from 43 to 116. Data were categorized by age class (adults, juveniles, and both) to determine how often rock hyraxes were inactive versus active and socializing versus isolating. We found that while rock hyraxes were primarily inactive and social year-round, they were more frequently observed active and isolating in the dry season compared to the wet season. Juveniles were primarily observed foraging, whereas adults were frequently seen resting alone in shady areas during hotter times of the day. These results suggest that the conditions in the dry season can significantly reduce rock hyrax social behavior and increase the amount of time spent in an active state, especially for juveniles. The increased energy budget of juveniles in this dry season study challenges previous studies which show that rock hyraxes are primarily inactive, thus warranting future research.

Keywords: rock hyrax, Tanzania, behavior, energy budget

1. Introduction

The rock hyrax (*Procavia capensis*) is a species of the family Procaviidae, the only extant family of the order Hyracoieda.[1] The rock hyrax lives in rocky areas of the savannah and can be found in sub-Saharan and northeast Africa, as well as the Middle East.[1] The rock hyrax is a gregarious, diurnal species, exhibiting social behavior and is primarily active during the day. As a result of these low energy expenditures while at rest, rock hyraxes spend most of their time in an inactive state.[2] Due to the rock hyrax's poor thermoregulation capabilities, they often bask or take refuge in rocky crevices to

stabilize their body temperature.[1] However, a study conducted in South Africa found that thermoregulation capabilities vary greatly between summer and winter, roughly corresponding to the wet and dry seasons, respectively.[3] Specifically, the study found that rock hyrax body temperature is lower during the winter but also more variable, likely because rock hyraxes spend more time basking to raise their body temperature. These results suggest that hyraxes have a lower energy budget during the dry season.

Rock hyraxes have a seasonal breeding period, usually during the dry season from August to November.[4] Males engage in significantly more territorial and aggressive behaviors such as calling and chasing during the breeding season, corresponding with significant increases in testicle size.[1] The presence of predators in an area influences the rock hyrax group size and the tendency to leave their refuges, meaning rock hyrax behavior is likely influenced by predators as well.[1] Rock hyrax predators include leopards, hyenas, jackals, and birds of prey.[5] These predators may exhibit different behavioral patterns at different times of year. A study of leopard population density in Serengeti National Park in Tanzania found that the population density was lower during the dry season than the wet season.[6] This pattern was concluded to be the result of leopards tending to avoid areas with other predators such as lions due to lower food availability during the dry season. As a result, rock hyrax behavior might differ between seasons based on seasonal variation in predator behavior.

These traits amongst others all relate to the rock hyrax's behavior and energy budget in some way, but this relationship has not been sufficiently studied in Tanzania. Rock hyraxes are among the least studied mammals in East Africa.[7] In 2024, Alice Thompson et al. conducted the first study on rock hyraxes in Randilen Wildlife Management Area (WMA) in Tanzania. However, it was conducted during a 10-day period in the wet season and therefore behavioral differences between wet and dry season have yet to be examined. Here, we aim to determine the seasonality of rock hyrax behavior and energy budget, as a comparative study between seasons in Randilen WMA is important for documenting variations depending on external environmental conditions. This information is pertinent to conservation, which has the potential to alter the rock hyrax's environment, energy budget, behavior, and midden composition.

Given that the rock hyrax spends most of its time in an inactive state and in the presence of other individuals,[1] we hypothesize a similar pattern will be observed in this study. In comparison to the wet season, rock hyraxes are expected to spend more time in an inactive state during the dry season because they will spend more time basking to regulate body temperature. The frequency of aggressive behaviors such as chasing, especially from males, are also expected to increase during the dry season, as the dry season coincides with the rock hyrax mating season, when intraspecies competition among male rock hyrax looking to reproduce increases.

2. Methods

2.1 Study Area Description

Randilen WMA is found in the Monduli District, one of seven districts in the Arusha Region of Tanzania. Randilen WMA occupies 312 km^2, or 31,200.68 ha, of land.[8] Randilen WMA borders Tarangire National Park to the south and the WMA member villages to the northwest, southwest, north, and east (Figure 1). The area contains multiple kopjes such as Buti, Kirowa, and Sunset Hills.[8] The climate is semi-arid and the area receives around 500-800 mm of precipitation annually. The short rains begin in November or December while the heavy rains begin from March to May.

The coordinates of the Buti Hill site are 3°42'34" S and 36°5'25" E (Figure 2). The study site

is an observation deck built at the top of a kopje, which is a suitable location to observe rock hyraxes due to their tendency to live in rocky areas with crevices. The kopje on which the observation area is situated overlooks Randilen and part of Tarangire.

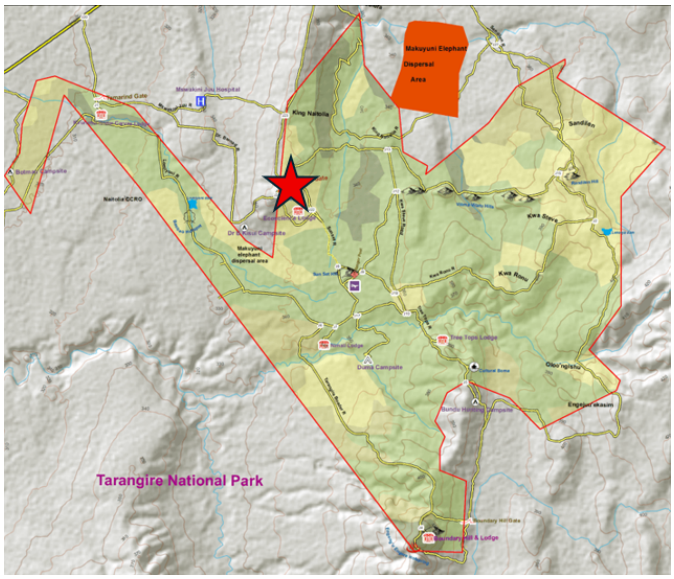

Figure 1. Map of Randilen WMA[9] showing the protected area boundary and the location of the main study site at Buti Hill marked by a red star.

Figure 2. Aerial view of Buti Hill at the main gate in Randilen WMA. The dropped pin indicates the coordinates of the fixed point of observation for the study.

2.2 Study Design

We collected data over a 10-day period from November 9th to November 18th, 2025. Mirroring Thompson et al.'s (2024) study, we collected the data in five hour intervals in the morning (6:00 to 11:00) and two hour intervals in the evening (17:00 to 19:00). We decided after the first day that scanning would end after 18:40 each evening instead of 19:00 due to the limited visibility after sunset at 18:30. In terms of energy budget, the rock hyrax 's activity state (i.e., energy budget) was categorized as either "Active" or "Inactive" and behavioral state as either "Socializing" or "Isolating". "Inactive" observations were those in which rock hyraxes were standing, sitting, or laying while "Active" observations were those in which rock hyraxes were running, walking, or climbing. "Socializing" and "Isolating" behaviors were determined by whether we observed a rock hyrax with or without the company of other rock hyraxes. Behaviors considered for this category were eating, basking, resting, huddling, playing, and mating.[2] None of the behaviors in Thompson et al.'s study are inherently active, so we categorized active rock hyraxes as either basking or resting depending on whether they were within direct sunlight while observed.

2.3 Methods

We observed the rock hyraxes from a fixed point on the observation deck and avoided causing disturbance by remaining as still as possible during data collection. Rock hyraxes did occasionally notice the observer when they entered the rock face during the earliest morning scans, but otherwise no disturbances were observed. We made ethograms during the preparation period to categorize rock hyrax behavior and activity state during the observation period (Supplementary Material 1). Distinctions between adult and juvenile rock hyraxes were made based on relative body size. Medium-sized rock hyraxes that appeared slightly larger than other juveniles were usually counted as juveniles.

We used scan sampling to make observations of the rock hyraxes. We conducted the scan from left to right every 5 minutes across all visible individuals from the observation point. Rock hyrax activity states and behaviors were recorded during each scan. Any observed behaviors not included in the predefined ethogram were noted.

The rock hyrax colony residing at the gate to Randilen was the sole subject of the study. The total area was 3,248 m^2 and the observable area was 1,578 m^2.[2] We found an estimated rock hyrax population on the kopje using the maximum number of hyraxes seen as well as the total area of the kopje and the area of observation (Equation 1). Using the highest maximum of 43, we estimated the population to be 116. Using the lowest maximum of 17, we estimated the population to be 46. Based on these calculations, the rock hyrax population size is estimated to range from 43 to 116. We utilized a t-test at the α=0.05 level to compare Thompson's wet season data to the new dry season data.

Equation 1. Species density estimate for the rock hyrax population on the kopje.[2] The equation multiplies the observed number of individuals from a certain species by the ratio of the total area to the observed area.

$$N_{est} = N_c(A_{tot}/A_c)$$

3. Results

3.1 Population

The results show 17 to 43 hyraxes observed each day in the dry season (Figure 3). We observed the highest maximum of 43 on Day 4 and the lowest maximum of 17 on Day 7. The wet season had a higher lowest maximum of 22 and lower highest maximum of 38. We observed a maximum of 9 to 25 juveniles each day in the dry season, and a similar range of 8 to 25 adults at this time. Despite the similar range, the mean maximum number of juveniles was 19 while the mean maximum number of adults was 15. In the wet season, the mean maximum number of juveniles was 23 while the mean maximum number of adults was 11.

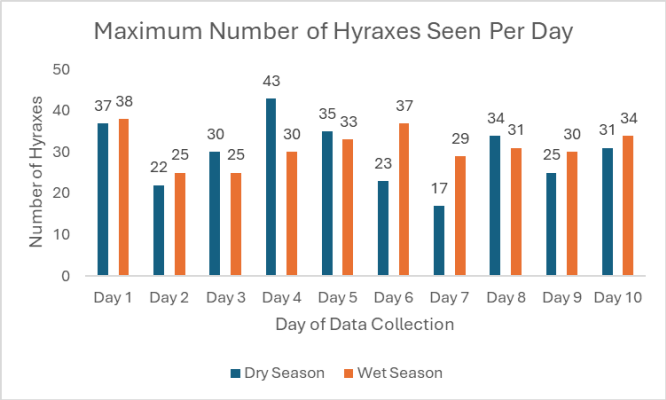

Figure 3. Maximum number of hyraxes seen during one scan on each day of observation in the dry and wet season, respectively (wet season data from Thompson et al., 2024).

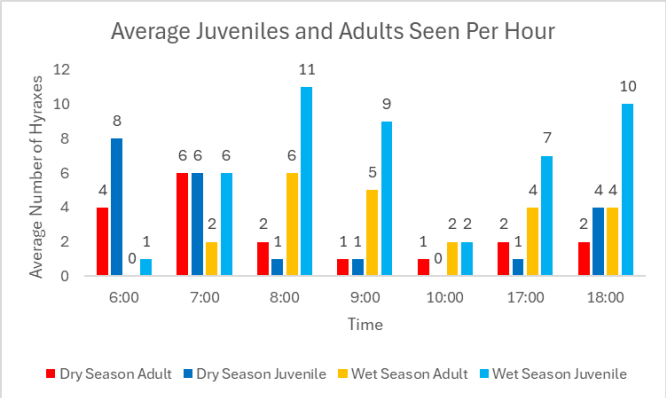

Figure 4. Combined graphs of the average number of juvenile and adult hyraxes seen on one scan during each hour of observation in the dry and wet season (wet season data from Thompson et al., 2024).

During the dry season morning scan of 6:00 to 11:00, we saw the most rock hyraxes during the hours of 6:00 and 7:00 with an average of 12 hyraxes per scan (Supplementary Material 2). During the dry season evening scan of 17:00 to 18:40, we saw the most rock hyraxes during the hour of 18:00. During the dry season morning scan, we saw the most juveniles during the hour of 6:00 and the most adults

during the hour of 7:00 (Figure 4). During the dry season evening scan we saw the most juveniles during the hour of 18:00 and we saw a similar number of adults during both hours. This adult data aligns with the wet season evening scan, but the most juveniles were seen during the hour of 18:00 in the wet season.

3.2 Behavior

We observed no instances of fighting at any time by any type of individual during the dry season. The combined data shows that rock hyraxes spent 78.12% of the observations socializing compared to 21.88% isolating in the dry season (Table 1). In the wet season, rock hyraxes spent 97.64% of the observations socializing, which is significantly greater than in the dry season study ($p < 0.0001$). Of the time spent socializing in the dry season, rock hyraxes spent 42.89% eating, 26.14% resting, 19.69% basking, 8.35% huddling, 2.88% playing, and 0.06% mating. Of the time spent isolating in the dry season, rock hyraxes spent 68.00% resting, 18.84% eating, and 13.16% basking.

Table 1. Summary of behavior for all hyraxes in the dry and wet season throughout the day, during the morning, and during the evening.

Combined Dry Season Data					
Energy Budget	100%	Inactive	52.90%	Sitting	72.54%
				Standing	5.67%
				Laying Down	21.79%
		Active	47.10%	Running	8.62%
				Walking	8.20%
				Climbing	83.18%
Morning Dry Season Data					
Energy Budget	100%	Inactive	54.83%	Sitting	78.70%
				Standing	5.10%
				Laying Down	16.20%
		Active	45.17%	Running	8.90%
				Walking	8.30%
				Climbing	82.80%
Evening Dry Season Data					
Energy Budget	100%	Inactive	45.43%	Sitting	43.75%
				Standing	8.33%
				Laying Down	47.92%
		Active	54.57%	Running	7.71%
				Walking	7.90%
				Climbing	84.39%

Combined Wet Season Data					
Energy Budget	100%	Inactive	76.87%	Sitting	21.61%
				Standing	18.56%
				Laying Down	59.83%
		Active	23.13%	Running	15.38%
				Walking	18.24%
				Climbing	66.38%
Morning Wet Season Data					
Energy Budget	100%	Inactive	74.95%	Sitting	25.94%
				Standing	15.00%
				Laying Down	59.06%
		Active	25.05%	Running	14.86%
				Walking	18.25%
				Climbing	66.89%
Evening Wet Season Data					
Energy Budget	100%	Inactive	80.54%	Sitting	14.89%
				Standing	24.08%
				Laying Down	61.02%
		Active	19.46%	Running	13.92%
				Walking	18.77%
				Climbing	67.31%

Adult rock hyraxes spent 69.60% of the observations socializing compared to 30.40% spent isolating in the dry season (Table 2). In the wet season, adult rock hyraxes spent 93.17% of the observations socializing. Based on a t-test, this observation is significantly greater than in the dry season study ($p < 0.0001$). Of the time spent socializing in the dry season, adult rock hyraxes spent 41.49% resting, 33.73% basking, 19.77% huddling, 4.57% eating, 0.31% playing, and 0.13% mating). Of the time spent isolating, adult rock hyraxes spent 79.51% resting, 15.33% basking, and 5.16% eating.

Table 2. Summary of behavior for adult hyraxes in the dry and wet season throughout the day, during the morning, and during the evening.

Combined Adult Data					
Energy Budget	100%	Inactive	87.58%	Sitting	70.14%
				Standing	4.61%
				Laying Down	25.25%
		Active	12.42%	Running	29.72%
				Walking	27.62%
				Climbing	42.66%
Morning Adult Data					
Energy Budget	100%	Inactive	88.50%	Sitting	76.32%
				Standing	4.55%
				Laying Down	19.13%
		Active	11.50%	Running	31.31%
				Walking	28.04%
				Climbing	40.65%
Evening Adult Data					
Energy Budget	100%	Inactive	83.67%	Sitting	42.55%
				Standing	4.88%
				Laying Down	52.57%
		Active	16.33%	Running	25.00%
				Walking	26.39%
				Climbing	48.61%

Combined Adult Data					
Energy Budget	100%	Inactive	77.40%	Sitting	26.19%
				Standing	16.91%
				Laying Down	56.91%
		Active	22.60%	Running	11.94%
				Walking	25.67%
				Climbing	62.39%
Morning Adult Data					
Energy Budget	100%	Inactive	76.50%	Sitting	29.13%
				Standing	15.12%
				Laying Down	55.76%
		Active	23.50%	Running	11.74%
				Walking	24.38%
				Climbing	63.88%
Evening Adult Data					
Energy Budget	100%	Inactive	78.98%	Sitting	21.22%
				Standing	19.93%
				Laying Down	58.85%
		Active	21.02%	Running	12.33%
				Walking	28.19%
				Climbing	59.47%

Juvenile rock hyraxes spent 86.93% of the observations socializing compared to 13.07% spent isolating in the dry season (Table 3). In the wet season, juvenile rock hyraxes spent 100% of the observations socializing. Based on a t-test, this observation is significantly greater than in the dry season study ($p < 0.0001$). Of the time spent socializing in the dry season, juvenile rock hyraxes spent 72.4% eating, 13.46% resting, 8.19% basking, 4.83% playing, and 1.38% huddling. Of the time spent isolating, juvenile rock hyraxes spent 50.16% eating, 41.64% resting, and 8.20% basking.

Table 3. Summary of behavior for juvenile hyraxes in the dry and wet season throughout the day, during the morning, and during the evening.

Combined Juvenile Data					
Energy Budget	100%	Inactive	19.11%	Sitting	85.43%
				Standing	9.87%
				Laying Down	4.71%
		Active	80.89%	Running	5.46%
				Walking	5.35%
				Climbing	89.19%
Morning Juvenile Data					
Energy Budget	100%	Inactive	20.40%	Sitting	89.49%
				Standing	7.55%
				Laying Down	2.96%
		Active	79.60%	Running	5.59%
				Walking	5.46%
				Climbing	88.95%
Evening Juvenile Data					
Energy Budget	100%	Inactive	14.56%	Sitting	65.33%
				Standing	21.33%
				Laying Down	13.33%
		Active	85.44%	Running	5.00%
				Walking	5.00%
				Climbing	90.00%

Combined Juvenile Data					
Energy Budget	100%	Inactive	76.63%	Sitting	19.13%
				Standing	19.46%
				Laying Down	61.42%
		Active	23.37%	Running	17.21%
				Walking	14.42%
				Climbing	68.60%
Morning Juvenile Data					
Energy Budget	100%	Inactive	74.05%	Sitting	24.12%
				Standing	14.93%
				Laying Down	60.95%
		Active	25.95%	Running	16.38%
				Walking	15.14%
				Climbing	68.25%
Evening Juvenile Data					
Energy Budget	100%	Inactive	80.81%	Sitting	11.73%
				Standing	26.16%
				Laying Down	62.11%
		Active	19.19%	Running	14.32%
				Walking	12.84%
				Climbing	69.38%

The frequency of socializing behavior in juveniles was higher than in adults. The most frequently observed behavior in adult rock hyraxes was socializing while resting, sometimes in trees but more often on the rocks, whereas the most frequently observed behavior in juvenile rock hyraxes was socializing while eating, typically in trees.

3.3 Energy Budget

Overall, rock hyraxes spent 52.90% of the observations in an inactive state compared to 47.10% in an active state in the dry season (Table 4). In the wet season, rock hyraxes spent 76.87% of the observations in an inactive state. Based on a t-test, this is significantly less than in the dry season study ($p < 0.0001$). Of the time spent in an inactive state in the dry season, rock hyraxes spent 72.54% sitting, 21.79% laying, and 5.67% standing. Of the time spent in an active state, rock hyraxes spent 83.18% climbing, 8.62% running, and 8.20% walking.

Table 4. Summary of energy budget for all hyraxes in the dry and wet season throughout the day, during the morning, and during the evening.

Combined Dry Season Data						Combined Wet Season Data					
Behavior	100%	Socializing	78.33%	Playing	2.84%	Behavior	100%	Socializing	97.64%	Playing	N/A
				Huddling	9.49%					Huddling	N/A
				Mating	0.06%					Mating	N/A
				Fighting	0.00%					Fighting	N/A
				Eating	42.36%					Eating	18.50%
				Basking	19.44%					Basking	76.75%
				Resting	25.81%					Resting	4.75%
		Isolating	21.67%	Eating	18.84%			Isolating	2.36%	Eating	13.51%
				Basking	13.16%					Basking	22.70%
				Resting	68.00%					Resting	63.78%
Morning Dry Season Data						Morning Wet Season Data					
Behavior	100%	Socializing	80.32%	Playing	3.35%	Behavior	100%	Socializing	96.35%	Playing	N/A
				Huddling	11.65%					Huddling	N/A
				Mating	0.07%					Mating	N/A
				Fighting	0.00%					Fighting	N/A
				Eating	39.78%					Eating	18.99%
				Basking	23.80%					Basking	77.72%
				Resting	21.36%					Resting	3.29%
		Isolating	19.68%	Eating	19.48%			Isolating	3.65%	Eating	14.12%
				Basking	15.47%					Basking	19.77%
				Resting	65.06%					Resting	66.10%
Evening Dry Season Data						Evening Wet Season Data					
Behavior	100%	Socializing	70.66%	Playing	0.60%	Behavior	100%	Socializing	99.73%	Playing	N/A
				Huddling	0.00%					Huddling	N/A
				Mating	0.00%					Mating	N/A
				Fighting	0.00%					Fighting	N/A
				Eating	53.72%					Eating	17.73%
				Basking	0.30%					Basking	75.22%
				Resting	45.39%					Resting	7.05%
		Isolating	29.34%	Eating	17.20%			Isolating	0.27%	Eating	0.00%
				Basking	7.17%					Basking	87.50%
				Resting	75.63%					Resting	12.50%

Adult rock hyraxes spent 87.58% of the observations in an inactive state compared to 12.42% in an active state in the dry season (Table 5). In the wet season, adult rock hyraxes spent 77.40% of the observations in an inactive state. Based on a t-test, this is significantly less than in the dry season study ($p < 0.0001$). Of the time spent in an inactive state in the dry season, adult rock hyraxes spent 70.14% sitting, 25.25% laying, and 4.61% standing. Of the time spent in an active state, adult rock hyraxes spent 42.66% climbing, 29.72% running, and 27.62% walking.

Table 5. Summary of energy budget for adult hyraxes in the dry and wet season throughout the day, during the morning, and during the evening.

Combined Adult Data						Combined Adult Data					
Behavior	100%	Socializing	69.60%	Playing	0.31%	Behavior	100%	Socializing	93.17%	Playing	N/A
				Huddling	19.77%					Huddling	N/A
				Mating	0.13%					Mating	N/A
				Fighting	0.00%					Fighting	N/A
				Eating	4.57%					Eating	18.73%
				Basking	33.73%					Basking	75.72%
				Resting	41.49%					Resting	5.54%
		Isolating	30.40%	Eating	5.16%			Isolating	6.83%	Eating	13.51%
				Basking	15.33%					Basking	22.70%
				Resting	79.51%					Resting	63.78%
Morning Adult Data						Morning Adult Data					
Behavior	100%	Socializing	73.15%	Playing	0.29%	Behavior	100%	Socializing	89.72%	Playing	N/A
				Huddling	23.20%					Huddling	N/A
				Mating	0.15%					Mating	N/A
				Fighting	0.00%					Fighting	N/A
				Eating	4.04%					Eating	17.75%
				Basking	39.43%					Basking	77.59%
				Resting	32.89%					Resting	4.66%
		Isolating	26.85%	Eating	6.00%			Isolating	10.28%	Eating	14.12%
				Basking	18.20%					Basking	19.77%
				Resting	75.80%					Resting	66.10%
Evening Adult Data						Evening Adult Data					
Behavior	100%	Socializing	54.38%	Playing	0.42%	Behavior	100%	Socializing	99.19%	Playing	N/A
				Huddling	0.00%					Huddling	N/A
				Mating	0.00%					Mating	N/A
				Fighting	0.00%					Fighting	N/A
				Eatting	7.63%					Eating	20.29%
				Basking	0.84%					Basking	72.78%
				Resting	91.10%					Resting	6.93%
		Isolating	45.62%	Eating	3.03%			Isolating	0.81%	Eating	0.00%
				Basking	8.08%					Basking	87.50%
				Resting	88.89%					Resting	12.50%

Juvenile rock hyraxes spent 80.89% of the observations in an active state compared to 19.11% in an inactive state in the dry season (Table 6). In the wet season, juvenile rock hyraxes spent 76.63% of the observations in an inactive state. Based on a t-test, this is significantly less than in the dry season study ($p < 0.0001$). Of the time spent in an inactive state in the dry season, juvenile rock hyraxes spent 85.43% sitting, 9.87% standing, and 4.71% laying. Of the time spent in an active state, juvenile rock hyraxes spent 89.19% climbing, 5.46% running, and 5.35% walking.

The frequency of an active state in juveniles was higher than in adults. Adults spent most of the

observations sitting, both in trees and on the rocks, whereas juveniles spent most of the observations climbing.

Table 6. Summary of energy budget for juvenile hyraxes in the dry and wet season throughout the day, during the morning, and during the evening.

Combined Juvenile Data						Combined Juvenile Data					
				Playing	4.83%					Playing	N/A
				Huddling	1.38%					Huddling	N/A
				Mating	0.00%					Mating	N/A
Behavior	100%	Socializing	86.93%	Fighting	0.00%	Behavior	100%	Socializing	100.00%	Fighting	N/A
				Easting	72.14%					Eating	18.39%
				Basking	8.19%					Basking	77.26%
				Resting	13.46%					Resting	4.36%
				Easting	50.16%					Eating	0.00%
		Isolating	13.07%	Basking	8.20%			Isolating	0.00%	Basking	0.00%
				Resting	41.64%					Resting	0.00%
Morning Juvenile Data						Morning Juvenile Data					
				Playing	5.97%					Playing	N/A
				Huddling	1.76%					Huddling	N/A
				Mating	0.00%					Mating	N/A
Behavior	100%	Socializing	87.67%	Fighting	0.00%	Behavior	100%	Socializing	100.00%	Fighting	N/A
				Easting	70.35%					Eating	19.60%
				Basking	10.43%					Basking	77.78%
				Resting	11.49%					Resting	2.62%
				Easting	49.55%					Eating	0.00%
		Isolating	12.33%	Basking	9.38%			Isolating	0.00%	Basking	0.00%
				Resting	41.07%					Resting	0.00%
Evening Juvenile Data						Evening Juvenile Data					
				Playing	0.69%					Playing	N/A
				Huddling	0.00%					Huddling	N/A
				Mating	0.00%					Mating	N/A
Behavior	100%	Socializing	84.33%	Fighting	0.00%	Behavior	100%	Socializing	100.00%	Fighting	N/A
				Easting	78.67%					Eating	16.47%
				Basking	0.00%					Basking	76.42%
				Resting	20.64%					Resting	7.10%
				Easting	51.85%					Eating	0.00%
		Isolating	15.67%	Basking	4.94%			Isolating	0.00%	Basking	0.00%
				Resting	43.21%					Resting	0.00%

4. Discussion

In the dry season, we saw the most rock hyraxes during the earliest two hours of the morning session and the last hour of the evening session. The wet season data showed more activity later in the morning and earlier in the evening, times when the temperature was higher relative to the rest of the day. Therefore, one possible reason for the lack of activity in the later hours of the morning during the dry season is the high temperatures. During this dry season study, the maximum temperature was between 31°C and 32°C. It is common for rock hyraxes to hide in crevices during hotter times of day.[1] These occurrences affected our visibility of rock hyrax behavior and might explain why so few rock hyraxes were seen during the later morning hours and the earliest evening hours in the dry season compared to the wet season.

According to the dry season observations, rock hyraxes spent 78.12% of the observations socializing compared to 21.88% of the observations isolating. Rock hyraxes are known to be social animals,[10] so this result is generally unsurprising. However, rock hyraxes, especially juveniles, spent significantly less of the observations socializing in this dry season study compared to the wet season study, in which juveniles were never observed to be isolated. Rock hyraxes tend to travel further from their homes to forage during the dry season,[10] which we observed during two instances where rock hyraxes were seen at the woodland near the bottom of the kopje. However, we observed most isolating behaviors at the top of the kopje and therefore cannot justify it with existing research. Despite the observations being conducted during the mating season, we observed mating only once during the 70 hours of observation in the dry season. These results are consistent with the wet season results, which was expected because the mating season occurs in the dry season in Randilen. In the dry season, the fact that the data was collected in November, at the end of mating season, suggests that most mating had already occurred for the year at the time of the study.[4] Similarly, we never observed fighting in the dry season. There were two instances in which adult rock hyraxes made loud vocalizations similar to those described in previous literature on male aggression during the mating season.[4] They were the only potential occurrences of aggressive behavior from males.

According to the dry season observations, rock hyraxes spent 52.90% of the observations in an inactive state compared to 47.10% of the observations in an active state. Contrary to the hypothesis that rock hyraxes would spend most of their energy budget thermoregulating by basking in the dry season, we observed resting in shady areas far more frequently. The rock hyraxes were still presumably thermoregulating, but they spent less time in direct sunlight than expected, especially in the later hours of the morning. Like the low numbers of rock hyraxes observed in the later hours

of the morning compared to the wet season, resting in the shade was likely the result of the much higher temperatures in the dry season discouraging the rock hyraxes from spending too much time in direct sunlight. During the later hours of the morning, we often observed a small number of rock hyraxes resting under a shaded part of a tree or on the kopje itself. The most common position in basking adults in the dry season was sitting, which differs from the wet season, where laying was most common. This difference might be explained by the temperature of the surface of the kopje being too warm for rock hyraxes to tolerate because of the high air temperature and direct sunlight in the dry season.

Rock hyraxes may have spent nearly the same amount of time in each activity state in the dry season primarily due to the relative inactivity of adults compared to juveniles. In the wet season, both adults and juveniles spent around three-fourths of the observations in an inactive state. The energy budget was relatively similar for adults between seasons, but juveniles spent significantly more of their energy budget in an active state in the dry season. By far the most common use of the juveniles' energy budget in the dry season was climbing, which was almost always paired with eating or playing. Rock hyraxes feed during a brief period in the morning three hours after sunrise and another brief period in the evening two hours before sunset.[4] The morning feeding period was earlier than what previous literature described, and the evening feeding period was later. However, there were two observable feeding periods less than an hour in duration each day. The juveniles observed in this dry season study almost exclusively ate while climbing trees, and eating while socializing was their most common behavior. There were up to 18 juveniles observed climbing and eating from a single tree on the kopje at a time. Other rock hyrax studies have shown that they primarily browse and eat from trees in the dry season,[11] which could explain the greater proportion of climbing observed during the dry season than the wet season. It is possible that the juvenile energy budget results were skewed by the large groups of juveniles being seen over several consecutive scans while other activities such as sitting and standing may have been conducted out of the viewing range. The larger proportion of climbing observed in juveniles in the dry season helps explain why they were more active than adults, but juveniles being primarily active is a drastically different finding from the wet season that has not yet been recorded in rock hyraxes.

Due to the rock hyraxes often being within or behind the foliage on the kopje and their coat color matching their surroundings, they were often hidden from our field of vision even when being looked at directly. As a result, it is possible that rock hyraxes were seen in one scan but not the next. This limitation in observations influenced the data for population size, behavior, and energy budget. Similarly, some rock hyraxes were of medium size and therefore difficult to categorize in terms of being juvenile or adult. Rock hyraxes of this size were usually considered juveniles, but it is possible that some of these hyraxes were adults and that the data for adults and juveniles was altered by this challenging distinction.

Additionally, only 70 hours of data were collected. This short timeframe limits the ability to draw wider conclusions about rock hyrax behavior and energy budget for the entire dry season. Furthermore, scan sampling has limitations as it does not continuously monitor behavior or track specific individuals. A continuous observation method would have provided further information on behavioral patterns which could help explain some of the findings of this study. The comparisons between the wet and dry season data may also be limited due to the definitions of certain behaviors and activity states, especially the difference between sitting and standing, not being standardized prior to data collection.

5. Conclusion

We found rock hyraxes to primarily spend their time socializing and in an inactive state in both the wet and dry seasons, but rock hyraxes were more frequently active and isolated in the dry season. Tanzania's dry season can be less bearable for the rock hyrax due to its higher temperatures and limited precipitation. The wet season data collection period saw lower maximum temperatures and more frequent precipitation.[2] The differences in weather conditions seemed to affect when the rock hyraxes were most observed as well as how often they basked as opposed to resting. We observed juvenile rock hyraxes climbing and eating more frequently in the dry season due to differing foraging habits between seasons. It is unknown why such a great proportion of juveniles were climbing and foraging in the dry season as opposed to exhibiting inactive behavior, but one possible hypothesis is that the juveniles foraged in areas that were visible to us and were inactive in areas outside of the viewing range.

This study found significant differences in rock hyrax energy behavior and energy budget between the wet and dry season that can be explained by the differing weather conditions and mating habits associated with each season. However, the findings obtained from this comparative study are limited by the lack of standardization between procedures. The wet and dry season studies had different interpretations of certain types of behavior and activity state because different researchers performed each study. It would be ideal for this study design to be conducted by a single team of researchers during both seasons to minimize variables between the two seasons or for reliability tests to be conducted between researchers.

Further research may be necessary to determine the underlying reasons for findings in this study. For example, it is unknown why rock hyraxes isolated more frequently in the dry season or why juveniles spent most of their energy budget climbing in the dry season. Neither of these findings have been observed in previous studies and they are not explainable by weather conditions. In the future, a study that captures a greater proportion of kopjes using cameras might be helpful in determining whether the results of this dry season study occurred because a great proportion of rock hyraxes were outside of the viewing range. If future findings are consistent with dry season study, it will be pertinent that behavioral studies are conducted to determine why isolating behavior and juvenile activity is more prevalent in the dry season in Randilen WMA and possibly beyond.

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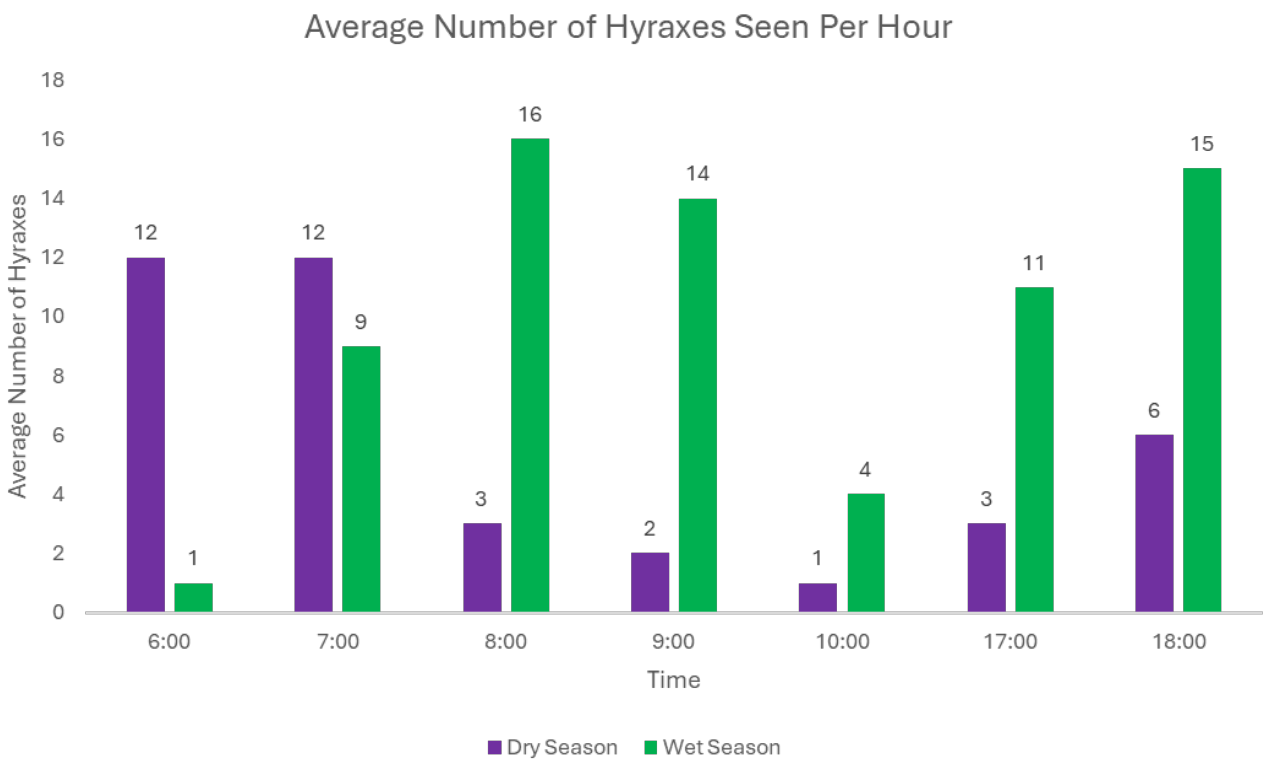
Appendix

Supplementary Material 1. List and description of energy states and behavioral states rock hyraxes can exhibit. All descriptions are based on personal observation.

Energy State		**Description**
Inactive	Sitting	Idle position in which the pelvis is in contact with the ground.
	Standing	Idle position in which the stomach is not in contact with the ground.
	Laying	Idle position in which the entire trunk, and sometimes the head and neck, is in contact with the ground.
Active	Running	An active state characterized by fast movement and synchronized locomotion between both of the front legs and both of the hind legs.
	Walking	An active state characterized by slower movement and no legs exhibiting synchronized locomotion.
	Climbing (vegetation)	Movement up or down a tree or other plant.
Behavior		**Description**
Socializing	Playing	The occurrence of multiple hyraxes running, jumping, chasing, or other active behaviors in a non-aggressive manner.
	Huddling	The occurrence of multiple inactive hyraxes (sitting, standing, or laying) being in contact with each other.
	Mating	The occurrence of one adult hyrax being mounted on another's back.
	Eating	The occurrence of a hyrax visibly chewing.
	Basking	The occurrence of a hyrax sitting or lying in an area with direct sunlight or in an area which would have direct sunlight in the case of cloudy weather.
	Resting	The occurrence of a hyrax sitting or lying in an area with shade or indirect sunlight.
	Fighting	The occurrence of multiple hyraxes running, jumping, chasing, calling, or making direct contact in an aggressive manner.
Isolating	Eating	The occurrence of a hyrax visibly chewing.
	Basking	The occurrence of a hyrax sitting or lying in an area with direct sunlight or in an area which would have direct sunlight in the case of cloudy weather.
	Resting	The occurrence of a hyrax sitting or lying in an area with shade or indirect sunlight.

Supplementary Material 2. Combined graphs of the average number of hyraxes seen on one scan during each hour of observation in the dry season (wet season data from Thompson, 2024).



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CAR-T Cell Therapies: An Emerging and Promising Treatment for Human Glioblastoma

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CAR-T Cell Therapies: An Emerging and Promising Treatment for Human Glioblastoma

Neha Nimma[1], Piper Levin[1], Emily Ricigliano[1], Sophia Shin[1], Leon Wang[1]

[1]Department of Human Science, Georgetown University, Washington, D.C., United States

Email: nn516@georgetown.edu
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Abstract

Human glioblastoma (GBM) is the most lethal type of brain tumor. Current therapies struggle to effectively target the tumor, leading to high recurrence rates and poor survival outcomes. Chimeric antigen receptor T-cell (CAR-T) therapy, although new and evolving, has shown success in hematological malignancies such as leukemia and lymphoma, and is currently being adapted for GBM. This review discusses emerging clinical and preclinical studies that serve as evidence for both the advancements and limitations of CAR-T therapy for GBM treatment. While prior reviews have addressed individual aspects of CAR-T therapy for GBM, this review explores how antigen selection, delivery strategy, and the tumor microenvironment collectively influence therapeutic outcomes. Tumor antigens have been used as therapeutic targets, each exhibiting differential expression patterns and distinct anti-tumor activity. Next-generation multivalent CAR-T constructs, which simultaneously engage multiple antigens or pathways, have shown promise in addressing GBM's heterogeneity and treatment resistance. Beyond antigen targets, delivery methods have also been found to play a crucial role in determining the success of this treatment. Cerebrospinal fluid and locoregional delivery show superior tumor penetration compared to typical intravenous delivery. Still, successful CAR-T engraftment faces major challenges, such as the immunosuppressive nature of the GBM microenvironment, the transient effects of CAR-T cells, and antigen loss. It is important to note that many findings discussed in this review are derived from small-scale Phase I clinical trials, which may limit the generalizability of the conclusions. Collectively, evidence indicates that CAR-T immunotherapy represents a promising, yet evolving, treatment with the potential to improve outcomes for patients with this malignant disease.

Keywords: glioblastoma, CAR-T cell therapy, immunotherapy, cancer, limitations, advancements

1. Introduction

Glioblastoma (GBM) is an extremely aggressive Grade IV astrocytoma that most commonly arises in the cerebral hemispheres.[1] Its highly infiltrative growth allows tumor cells to migrate into surrounding healthy brain tissue, making complete surgical resection nearly impossible. Although relatively rare, GBM accounts for 54% of all gliomas and 16% of all primary brain tumors in the United States, with an incidence of approximately 3.2 per 100,000 people annually.[1] GBM resides within the blood-brain barrier (BBB), rendering many forms of

chemotherapy useless, as they are unable to reach the tumor. The BBB is a highly selective membrane formed by specialized endothelial cells lining brain blood vessels. Its tight junctions act as a protective filter, preventing pathogens and most foreign substances from entering the brain. While this is essential for neurological protection, it also means that most chemotherapy drugs, which must enter the bloodstream to travel to the brain, are blocked from reaching the tumor. Additionally, no standard therapies at relapse have shown any survival benefit in clinical trials.[2] Together, infiltrative growth, BBB restriction, and rapid molecular adaptation make GBM highly resistant to conventional therapies and contribute to its high recurrence rate.[3] Despite multimodal treatment such as surgery, radiation, and chemotherapy, prognosis remains poor, with median survival under 15 months.[4] However, emerging immunotherapies show promise for glioblastoma treatment. Immunotherapies seek to change the immune response by using the patient's own immune system to recognize and kill cancer cells. Recently, a promising immunotherapy, CAR-T cell therapy, has been explored for GBM treatment. CAR-T cells are generated by genetically engineering a patient's T cells to express synthetic receptors that enhance antigen recognition, activate cytotoxic signaling pathways, and promote targeted tumor cell elimination. The therapy has shown great success in blood cancers such as leukemia and lymphoma, and is now expanding to solid tumors, including glioblastoma.

However, translating CAR-T therapy to GBM presents challenges not encountered in blood cancers. Circulating tumor cells in leukemia and lymphoma are readily accessible to infused CAR-T cells, whereas GBM resides behind the BB and within a hostile tumor microenvironment (TME). The TME is immunosuppressive and can inhibit CAR-T cell function through multiple mechanisms, thereby contributing to tumor resistance to therapy. These barriers limit CAR-T trafficking and functional activity. Additionally, GBM exhibits substantial intratumoral heterogeneity, which enables antigen-negative tumor cells to evade monospecific CAR-T constructs and cause relapse. Clonal evolution, the process by which tumor cells acquire new mutations over time, further contributes to this heterogeneity by allowing antigen-negative variants to gain a survival advantage and selectively proliferate. To address these obstacles, recent preclinical and early-phase clinical studies have explored multivalent CAR designs, new antigen targets, and locoregional delivery strategies such as intratumoral, intracerebroventricular, and intrathecal administration. These approaches aim to reduce antigen escape and enhance CAR-T cell function within the TME.

This review synthesizes emerging evidence from early-phase clinical and preclinical studies to evaluate how antigen selection, delivery methods, and constructs that influence the microenvironment can collectively shape the current trajectory for GBM treatment.

2. Antigen Targets and Multivalent/Multi-Pathway CAR-T Designs

2.1 IL-13Rα2 Targeting

Interleukin-13 receptor alpha-2 (IL-13Rα2)-directed CAR-T cell therapy represents a targeted immunotherapeutic approach for GBM, exploiting the tumor's overexpression of IL-13Rα2 to achieve antitumor activity while minimizing off-target effects on healthy brain tissue. Brown et al. (2022) marked a meaningful advance in IL-13Rα2-targeted CAR-T cells for glioblastoma by engineering a steroid-resistant, allogeneic product, GRm13Z40-2.[5] Using zinc-finger nucleases to disrupt the glucocorticoid receptor, donor-derived CAR-T cells were created that retained their effector function. This product was designed to be manufacturable as an "off-the-shelf" therapy,

meaning it can be produced in advance from donor cells and stored for use in any patient, rather than requiring individualized manufacturing from each patient's own cells. This distinction is clinically significant because personalized manufacturing is costly, time-consuming, and not always feasible for patients with rapidly progressing disease. Their first human Phase I Trial (n = 6) demonstrated that intracranial delivery of GRm13Z40-2 was safe, exhibiting no signs of alloreactivity, and showed transient antitumor activity despite ongoing steroid therapy, including tumor necrosis at infusion sites. While promising, the extremely small sample size of 6 patients limits the conclusions that can be drawn from this study. However, the study proved the feasibility of a therapy that is both manufacturable as "off-the-shelf" and functionally resilient in an immunosuppressive tumor environment.[5] This is a significant discovery given the steroid sensitivity of many previous CAR-T cell therapies.

Building on the progress of the 2022 study, Brown et al. (2024) published the findings of a Phase I trial with 65 patients experiencing recurrent high-grade glioma.[6] In this trial, locoregional delivery, the administration of a treatment directly to the tumor or specific area of the body, of IL-13Rα2-targeted CAR-T cells proved to be safe and feasible (Brown et al., 2024). No dose-limiting toxicities were observed, and a clinical maximum feasible dose of 200×10^6 CAR-T cells per infusion cycle was reached. In approximately 50% of evaluable patients, the disease stabilized or improved, including two partial responses and two complete responses. Moreover, patients in the optimized dual intratumoral/ intraventricular CAR-T cell delivery group experienced a median overall survival of 10.2 months, exceeding the 7.7-month median for the overall cohort.[6] These findings establish that IL-13Rα2 is a viable antigen target in human glioma, and that the locoregional delivery method can prevent some of the toxicity and delivery challenges.

Together, these studies demonstrate progressive advances in IL-13Rα2 CAR-T development by establishing steroid-resistant, off-the-shelf feasibility and confirming safety and early signs of clinical activity in a larger patient cohort. IL-13Rα2 CAR-T development exemplifies how advances in antigen targeting and CAR-T cell engineering can work in tandem to improve the feasibility, safety, and efficacy of CAR-T cell therapies in human glioblastomas. However, the transient nature of responses in both studies highlights the continued need for further research and clinical trials to establish long-term efficacy and nontoxicity.

2.2 EGFR / EGFRvIII Targeting

Epidermal Growth Factor Receptor variant III (EGFRvIII) is a promising therapeutic tumor-specific target antigen for GBM treatment by CAR-T cell technology. EGFRvIII is expressed in approximately 40% of GBM cases, producing a tumor-specific, oncogenic, and immunogenic epitope.[7,8] Being entirely absent from healthy tissue, EGFRvIII offers a unique opportunity for potent antitumor activity without the risk of off-target toxicity. First in-human clinical trials confirm that EGFRvIII-directed CAR-T cells are safe and feasible, typically avoiding severe cytokine release syndrome and off-target effects.[7] The lack of side effects associated with this therapy demonstrates the selective nature of the EGFRvIII target. Nonetheless, the therapeutic impact of this treatment is limited by antigen escape, driven by the clonal evolution characteristic of GBM. EGFRvIII expression frequently occurs in a mosaic against non-mutated EGFR amplification, allowing antigen-negative cells to evade T-cell detection and driving disease recurrence.[8] Once refined to account for clonal evolution, researchers can leverage this specificity to initiate more durable and effective tumor clearance.

To overcome GBM intratumoral heterogeneity, researchers have developed more advanced CAR-T designs. While first- through third-generation CAR-T cells mainly focused on improving T-cell activation, fourth-generation "armored" CAR-T cells are engineered to secrete immune-modulating proteins such as signal regulatory protein gamma-related protein (SGRP). EGFRvIII-directed CAR-T cells secreting SGRP trigger phagocytosis in both antigen-positive and antigen-negative tumor cells, overcoming the inherent heterogeneity of GBM.[8] Phagocytosis is the result of immune modulation via SGRP, which shifts the typically immunosuppressive tumor microenvironment to immunostimulatory by utilizing EGFRvIII recognition to kill antigen-positive cells and SGRP secretion to kill bystander cells. Administration of this treatment resulted in nearly complete eradication of EGFRvIII-mosaic GBM, highlighting the clinical potential of reprogramming the immunosuppressive TME to facilitate comprehensive tumor regression.[8] Consequently, SGRP-secreting EGFRvIII CAR-T cells address the limitations of monovalent EGFRvIII therapies by providing a strategy that achieves a sustained therapeutic response against heterogeneous GBM. Together, these advances reaffirm the potential for EGFRvIII as a clinically viable and safe target antigen for GBM, but still require next-generation designs such as SGRP-secreting constructs to achieve durable responses. Coupled with immune-modulatory designs such as SGRP secretion, EGFRvIII-directed CAR-T cells can overcome antigen escape and drive comprehensive and sustained tumor regression in heterogeneous GBM.

2.3 GD2 Targeting

GD2-antigen CAR is a relatively new and developing therapy, and current studies are only preclinical. GD2 is a disialoganglioside normally expressed at low levels in healthy tissues but highly upregulated in several solid tumors, including glioblastoma. Its restricted expression pattern and association with aggressive tumor behavior offer potential for selective tumor recognition. A pre-clinical study conducted by Prapa et al. investigated GD2-targeting CAR-T cells, which demonstrated strong antitumor activity in both two-dimensional and three-dimensional glioblastoma models. The study tested cells from 12 patient-derived samples, with 7 of 12 showing GD2 positivity greater than 80%.[4] Most importantly, the GD2 CAR-T cells produced dramatic anticancer effects with clusters of activated cytotoxic lymphocytes. In this study, TGF-β1 served as a test of whether the immunosuppressive tumor microenvironment could impair GD2 CAR-T cell function. TGF-β1 is an immunosuppressive cytokine abundantly secreted by glioblastoma cells and tumor-associated cells. These proteins usually pose a challenge to CAR-T efficacy, contributing to immunosuppression and preventing the CAR-T cells from killing tumor cells. However, in the Prapa et al. study, TGF-β1 did not have an impact on the treatment's antitumor activity.[4] TGF-β1 was consistently expressed in all culture conditions at both 48 hours and 7 days.

Despite this immunosuppressive environment, GD2-directed CAR-T cells maintained significant killing activity, a finding that Prapa et al. suggest may reflect the inherently strong activation profile of GD2 CAR-T cells and their ability to sustain cytotoxic signaling even in the presence of TGF-β. This resistance may also relate to the high levels of granzyme B and pro-inflammatory cytokines produced by the GD2 CAR-T cells, which can counteract TGF-β–mediated suppression.[4] Across studies, multivalent designs consistently outperform monovalent constructs in preventing antigen escape, suggesting that multi-targeting is becoming a foundational requirement for GBM CAR-T therapy. GD2 targeting remains at the pre-clinical stage and has not yet entered formal clinical trials in GBM patients; clinical translation will require safety and

toxicity testing in early-phase human trials before broader use can be considered.

2.4 Bivalent and Multivalent CAR Constructs

There have been recent critical advancements regarding the number of antigens a CAR-T cell targets, with both bivalent (targeting two antigens) and multivalent (targeting more than two antigens) constructs showing promising results in early phase studies. Bagley et al. showcase a bivalent CAR construct in which the two antigens previously discussed in Sections 2.1 and 2.2 are targeted simultaneously by a single CAR-T cell.[9] Beyond enhancing targeting efficacy, the study's findings suggest that the bivalent design facilitated wider CAR-T cell expansion and distribution, as evidenced by their presence in the CSF and peripheral blood in addition to the injection site. Most notably, the impact of these constructs on the development of CAR-T cell therapy is their ability to overcome the challenge of antigen loss.

2.5 dnTGFβRII Suppression Prevention

Unlike bivalent or multivalent constructs that expand antigen recognition, CART-EGFR-IL13Rα2 cells use a dominant-negative TGF-β receptor II (dnTGFβRII) domain to resist TGF-β–driven immunosuppression, allowing the CAR-T cells to remain active in the GBM microenvironment. The study reported by Li et al. shows potential solutions to barriers caused by TGF-β. A dominant-negative TGF-β receptor II (dnTGFβRII) domain was incorporated into the CART-EGFR-IL13Rα2 construct.[10] This modification does not expand antigen targeting; instead, it blocks TGF-β signaling, preventing CAR-T cells from receiving suppressive signals within the tumor microenvironment. Li et al. reported enhanced tumor killing along with increased effector cytokine production and improved T-cell proliferation, indicating that with dnTGFβRII, the CAR-T cells remained active despite high TGF-β levels.[10] This demonstrates how CAR-T-EGFR-IL13Rα2 cells with dnTGFβRII can overcome immunosuppression, highlighting the potential of multivalent CAR constructs.

3. Delivery Methods and Their Impact on Efficacy

3.1 Intravenous Delivery

Intravenous (IV) administration is the most clinically practical and used delivery route for CAR-T therapy. A first-in-human study by O'Rourke et al. demonstrated that IV delivery of EGFRvIII-directed CAR-T cells in ten recurrent glioblastoma patients was safe and feasible, with no evidence of off-tumor toxicity or cytokine release syndrome.[7] The study confirmed successful penetration of the BBB and local expansion within GBM. However, even though all patients infused with the CAR-T therapy had detectable CART-EGFRvIII cell engraftment, several significant limitations emerged. CART-EGFRvIII cells engrafted approximately 50-fold less than CD19-specific CAR-T cells for leukemia and other blood cancers using the same manufacturing process.[7] This reflects the challenge of target antigens being confined to brain tissue rather than circulating throughout the blood and lymphoid organs. Second, CAR-T trafficking to the tumor was limited, with relatively low numbers of cells detected in peripheral blood and cerebrospinal fluid compared to expectations from systemic CAR-T therapies. Finally, post-infusion tumor specimens showed increased expression of immunosuppressive molecules and reduced CAR-T cell persistence, along with decreased EGFRvIII expression after 30 days; this is an indication of both microenvironment-mediated suppression and antigen loss.[7] These findings highlight how IV delivery faces challenges of limited rapid trafficking to target tumor areas and tumor microenvironment resistance.

3.2 Locoregional Delivery: Intracerebroventricular (ICV), Intrathecal (IT), Intracranial (IC)

Recent clinical trials have shown that CSF-based delivery routes such as intracerebroventricular (ICV), intrathecal (IT), and intracranial (IC) administration offer promising advantages for GBM treatment in comparison to IV routes. Brown et al. (2024) conducted a Phase I clinical trial with IL-13Rα2-targeted CAR-T cells for recurrent high-grade GBM with locoregional CAR-T delivery testing intratumoral (ICT), ICV, and dual delivery.[6] ICT delivery is the direct injection of CAR-T cells into the tumor site through a catheter placed during surgery. This can maximize local concentration of CAR-T cells at the primary tumor site and minimize systemic exposure; however, it may not effectively target tumor cells that have migrated away from the primary site. ICV application delivers CAR-T cells into the cerebral ventricles, allowing the cells to circulate throughout the CSF, providing broader coverage of the central nervous system. However, using ICV treatment alone can cause large intraparenchymal tumors deep within the brain tissue to continue to progress while just eliminating small subpial lesions.[6]

Because ICT was beneficial for eradicating unifocal (single-location) tumors and ICV was more beneficial for multifocal (multiple-location) tumors, the dual approach was administered to overcome individual limitations by getting cells to both large, deep, and multifocal tumors. After the administration of the dual therapy, CAR-T cells were detected in CSF and tumor cavity fluid for the majority of patients for more than 7 days post-infusion, which is significant since CSF volume turns over approximately four times.[6]

The efficacy of locoregional delivery was also demonstrated in Bagley et al.'s intrathecal bivalent CAR-T trial, which administered the CAR-T cells directly into the CSF. Peaks of CAR-T cells in CSF showed similar levels to those in hematologic cancers treated with CD19 CAR-T cells.[9] Additionally, reductions in tumor enhancement and size were observed in all six patients on early MRI, with tumor shrinkage of at least 30% in three of six patients. Neurotoxicity side effects did arise, but were controllable with high-dose dexamethasone and anakinra.[9]

Chan et al.'s systematic review of 13 trials covering 128 patients with IV, ICV, ICT, and intracavitary deliveries confirmed this safety profile: the direct methods of CAR-T therapies were well tolerated with low dose-limiting toxicities. The review also highlighted advantages of the direct delivery method with CAR-T cells in blood being consistently lower (0%-2% concentration) than in CSF (~70% concentration) after intraventricular injection.[11] This is in comparison to the intravenous-only study that detected little to no CAR-T cells in the brain. However, one notable limitation is discussed in a study by Choi et al., which tested CARv3-TEAM-E, a therapy that targets two types of EGFR: the tumor-specific variant and the normal protein. With direct CSF application, there was a report of the development of anti-CARv3-TEAM-E antibodies in the CSF, which increased with each reinfusion, leading to immunogenicity barriers.[12] However, these results may only be attributed to that specific CAR-T therapy and associated antigens.

In summation, these findings establish a growing foundation that locoregional delivery significantly improves tumor-site CAR-T concentration while maintaining manageable toxicity. This leads to a pattern in which CSF-based routes become the preferred approaches for GBM CAR-T therapy over IV delivery, which is limited by trafficking inefficiency. A comparative overview of IV, ICV, and ICT/IT delivery routes is summarized in Table 1, which highlights key differences in BBB penetration, tumor-site exposure, toxicity profiles, and CAR-T persistence.

Table 1. Comparative Characteristics of Intravenous, Intraventricular, and Intratumoral/Intrathecal CAR-T Delivery Routes for GBM

Parameter	IV (intravenous)	ICV (intraventricular)	ICT/IT (intratumoral/intrathecal)
BBB Penetration	Poor Blocked by BBB; limited entry	Good Bypasses BBB via ventricles	Excellent Direct CNS/tumor placement)
Tumor-Site Concentration	~0-2% in CSF 50x less than blood cancer IV CAR-T	~70% in CSF High CSF levels post-injection	Maximized locally Comparable to CD19 CAR-T (IT)
Coverage Scope	Systemic Whole body; poor CNS targeting	Broad CNS Multifocal; may miss post-injection	Focal/Broad CNS ICT focal; IT broad CSF coverage
Adverse Effects	Low (no CRS observed) Limited CAR-T expansion	Higher (grade 3+ reported)	Low-Moderate CRS grade 1-2; no dose-limiting toxicities
CAR-T Persistence	Transient Undetectable in blood after 30 days	Moderate CSF detection varies by case	Good (>7 days in CSF) Detected beyond injection site (ICT)
Clinical Suitability	Limited use in GBM; poor CNS penetration	Best for multifocal/disseminated disease; combine with ICT	Preferred route; dual ICT+ICV approach improves Overall survival to 10.2 months

Sources: Brown et al. (2024); O'Rourke et al. (2017); Bagley et. al (2024); Chan et al (2025); Choi et al. (2025)

4. Safety Considerations

4.1 Cytokine Release Syndrome (CRS) and Neurotoxicity

Two major adverse effects of CAR-T cell therapy in patients are cytokine release syndrome (CRS) and neurotoxicity. Notably, in a pilot study by Bagley et al., all 6 patients enrolled in the Phase I trial developed CRS, and all the cases were classified as low-grade based on the American Society for Transplantation and Cellular Therapy criteria.[9] The patients who received a lower dosage exhibited grade 1 CRS, while the patients who received a higher dosage exhibited both grade 1 and grade 2 CRS. Unfortunately, all 6 patients also developed moderate-severe neurotoxicity, graded using a modified Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) severity scale. Dose variations, however, did not result in any meaningful differences in the severity of the neurotoxicity developed. This study utilized an intrathecal method of delivery: administration of the therapy into the CSF (as opposed to the bloodstream) means there is limited circulation of the CAR-T cells. A reduced exposure decreases the likelihood of CRS in peripheral blood. Ultimately, this highlights how the route of delivery can greatly influence the adverse events experienced by the

patients.[9] In another study by O'Rourke et al., patients did not develop systemic CRS or EGFR-directed toxicity.[7] Because this trial used intravenous delivery, the absence of toxicity cannot be attributed solely to the route of administration. Instead, the low toxicity is more likely explained by the biology of the target and the behavior of the CAR-T cells. EGFRvIII is expressed only on tumor cells, not on normal tissues, and the CAR-T cells in this study showed limited expansion. Together, these factors reduce the likelihood of off-tumor toxicity.

4.2 Off-Target Effects and Toxicity Profiles

In an analysis of 13 Phase I trials by Chan et al., where CAR-T cell therapy was administered in patients with GBM, dose-limiting toxicities were noted at relatively higher doses from 1×10^7 to 1×10^{10} cells.[11] This is further illustrated by how six out of the seven studies reporting adverse effects of grade three or higher used dose orders of 1×10^7 and above. Regarding delivery methods of the CAR T cell therapy, Chan et al. concluded that the most adverse effects observed per patient (beyond CRS and neurotoxicity) were therapies involving an intraventricular injection of CAR-T cells targeted at the EGFR and IL-13 antigens.[11] On the contrary, a study by Brown et al. concluded that there were no dose-limiting toxicities observed with a locoregional delivery of CAR-T cells.[6] Similarly, a study by Barish et al. also found that there were no limiting toxicities observed with Chlorotoxin-directed CAR-T cell therapy, as well as no development of antibodies to the treatment.[13]

5. Summary: Overall Clinical Progress

Selectively overexpressed surface antigens such as IL-13Rα2, EGFRvIII, and GD2 have emerged as promising targets for CAR-T cell therapy for GBM, demonstrating significant clinical efficacy in early-stage clinical trials. Among these, IL-13Rα2 stands out due to its demonstrated clinical feasibility, including off-the-shelf potential, steroid resistance, and compatibility with a locoregional delivery approach.[5,6] EGFRvIII remains a highly specific tumor-restricted target, and next-generation constructs engineered to secrete SGRP have shown enhanced activity against heterogeneous tumors.[8] GD2, while still in pre-clinical development, is consistently expressed on aggressive glioma cells and has demonstrated potent antitumor activity even under TGF-β–rich conditions.[4] Together, these antigens are complementary strategies for targeting both bulk and resistant tumors. A persistent challenge across all antigen-specific approaches is antigen loss and escape, which limits the durability of mono-specific CAR-T responses. Strategies such as SGRP secretion, multivalent targeting, and constructs resistant to TGF-β–mediated suppression can counteract this problem by enabling CAR-T cells to eliminate both antigen-positive and antigen-negative tumor cells.[8] Despite these advances, long-term persistence remains limited, with evidence of T-cell exhaustion and reduced proliferation over time.[6]

To address GBM's cellular heterogeneity, bivalent and multivalent CAR-T constructs have shown improved efficacy over monovalent designs by simultaneously recognizing multiple antigens.[9] These constructs demonstrate broader tumor coverage and improved resistance to antigen escape. Delivery route is also another critical determinant of therapeutic success. IV administration, while clinically convenient, is hindered by the BBB, limited trafficking, and microenvironment-mediated suppression.[7] In contrast, locoregional delivery such as ICT, ICV, and IT routes achieve higher local CAR-T concentration, reduced systemic toxicity, and improved persistence.[14] Optimizing delivery strategies is an essential step in translating CAR-T potency into more clinical trials.

Across studies, a major barrier to progress is the lack of standardized patient cohorts and reporting formats. Variability in eligibility criteria, dosing schedules, and outcome reporting complicates cross-trial comparisons and limits the ability to draw reliable conclusions.[10,11] Greater standardization to reduce confounding variables and improve interpretability. In terms of clinical translation, IL-13Rα2-targeted CAR-T cells are the most advanced, now progressing toward Phase II evaluation. EGFRvIII constructs, particularly SGRP-secreting variants, are entering later-stage testing, while GD2- and dnTGFβRII-based therapies remain in pre-clinical development. If Phase II trials confirm safety and early efficacy, regulatory approval for next-generation constructs could be feasible within the next 5–10 years; though this is contingent on consistent outcomes and scalable manufacturing. Overall, the field is moving toward integrating multi-mechanism CAR-T strategies that simultaneously address antigen heterogeneity, microenvironment suppression, and delivery barriers. This approach appears most likely to yield stronger clinical responses for GBM.

6. Conclusion

Recent clinical and preclinical studies demonstrate that CAR-T therapy for GBM is progressing toward more effective and durable strategies through advancements in antigen targeting, delivery optimization, and anti-suppressive microenvironment designs. Early trials involving IL-13Rα2, EGFRvIII, GD2, and multivalent constructs show that CAR-T cells can safely target GBM through overcoming the challenges posed by the BBB and the immunosuppressive tumor microenvironment. Moving forward, the most promising progress will come from integrated approaches that combine multi-antigen recognition, enhanced persistence and capacity mechanisms, and resistance to microenvironmental suppression. More clinical trials using locoregional delivery methods is essential for maximizing tumor exposure while minimizing systemic toxicity. Equally important is the need for standardized clinical trial designs, which will enable clearer comparisons across studies and accelerate the path toward regulatory approval.

Although challenges remain, including antigen escape, limited persistence, and risks such as cytokine release syndrome and neurotoxicity, emerging evidence supports CAR-T therapy as an increasingly viable option for GBM. By combining antigen selection, delivery strategy, and microenvironment engineering into one cohesive treatment, next-generation CAR-T constructs hold significant potential to improve outcomes for patients with this malignant and treatment-resistant disease.

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The Use of Non-pharmacological Interventions to Treat Cognitive Dysfunction Following Traumatic Brain Injuries

Ahursa Maryam Ghalebi, Shruthy Thamizhavel,
Natalie Carousso, Viviana Méndez Vélez

The Use of Non-pharmacological Interventions to Treat Cognitive Dysfunction Following Traumatic Brain Injuries

Ahursa Maryam Ghalebi[1], Shruthy Thamizhavel[1], Natalie Carousso[1], Viviana Méndez Vélez[1]

[1] Department of Human Science, Georgetown University, Washington, D.C., United States

Email: ahursamaryam@gmail.com
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Abstract

A traumatic brain injury (TBI) triggers a complex cascade of biological changes following a forceful impact to the head. Long-term effects are often life-altering and include cognitive impairment, reduced synaptic plasticity, mood instability, and motor dysfunction. Current pharmacological treatments are limited in their ability to address the evolving and multifaceted nature of TBI pathology and may carry adverse side effects or risk of dependence. This literature review synthesizes emerging evidence supporting non-pharmacological interventions (NPIs) that are currently in their clinical trial phase as complementary or alternative approaches. Virtual reality (VR), hyperbaric oxygen therapy (HBOT), nutritional therapy, and exercise-based interventions demonstrate promising early results. VR therapy has been shown to improve cognitive function and mood, particularly when combined with physical rehabilitation to enhance balance and mobility. HBOT may mitigate secondary injury processes, including hematoma progression, and improve pain outcomes. Nutritional interventions are associated with improved cognitive function and earlier hospital discharge, while exercise-based therapy supports executive function and allows for individualized rehabilitation strategies. Together, these findings suggest that NPIs may enhance post-TBI care when integrated with pharmacological treatment within personalized rehabilitation models. However, larger controlled studies are needed to confirm long-term efficacy and clinical feasibility.

Keywords: traumatic brain injury, cognitive recovery, non-pharmacological interventions, aerobic exercise, virtual reality, hyperbaric oxygen therapy

1. Introduction

Traumatic brain injury (TBI) is the primary source of mortality and impairment in non-elderly groups in industrialized countries.[1] It is defined as neurological injury resulting from external mechanical force. Patients with TBI experience debilitating cognitive symptoms that hinder their quality of life, including decreased language abilities, reasoning skills, and executive function limitations. In the United States, TBIs were responsible for approximately 214,110 hospitalizations in 2020 and 69,473 related deaths in 2021.[2] Beyond the initial injury, TBI can cause long-term symptoms including post-traumatic epilepsy (PTE), post-traumatic stress disorder (PTSD), and post-traumatic cognitive impairment (PTCI). Among

these, cognitive dysfunction is one of the most common and weakening outcomes. Patients may experience difficulties with memory, attention, emotional regulation, language, reasoning, and executive function, all of which can affect quality of life. Memory impairments have been reported in up to 90% of cases, attentional difficulties in 82%, and executive dysfunction in 75% of TBI populations.[3] Additionally, persistent cognitive impairment occurs in approximately 65% of patients with moderate to severe TBI and 15% of patients with mild TBI.[3] These deficits persist long after the initial injury, making cognitive recovery an important focus of TBI treatment.

The standard of care for pharmacological management of cognitive dysfunction following TBI is ever-evolving as more drugs are developed and tested for their efficacy in treating the various cognitive deficits following TBI. Some common drugs used to support cognitive recovery include methylphenidate, donepezil, and amantadine. Although pharmacological interventions are standardly used to support cognitive recovery following TBI, many of these interventions demonstrate inconsistent efficacy, limited long-term benefit, or adverse side effects, highlighting the need to investigate alternative treatments.[3,4] Methylphenidate has little evidence to support a strong dose-response relationship.[5] Donepezil has been shown to have a partially safe and tolerable profile with chances of harmful quality of life side effects such as diarrhea, nausea, and dehydration.[6] Amantadine has been shown to have unreliable effects, improving functional recovery in some patients but not others, with risks of adverse side effects such as seizures and gastrointestinal problems.[7]

The purpose of this review is to assess non-pharmacological interventions (NPIs), or treatment approaches that do not involve medications for TBIs. Despite growing interest in NPIs for TBI, existing literature reviews largely examine these interventions in isolation, limiting cross-modal comparison and mechanistic integration. This review addresses that gap by comparatively synthesizing virtual reality (VR), hyperbaric oxygen therapy (HBOT), nutritional therapy, and exercise-based rehabilitation through a comparative, mechanism-informed approach. By evaluating how these distinct modalities may influence common recovery processes, including neuroplasticity, cerebral oxygenation, metabolic stabilization, and functional recovery, this review highlights the potential of these NPIs to be combined with pharmacological treatments.

1.1 Limitations of Pharmacological Interventions

While the current TBI-related pharmacological interventions have been shown to improve some aspects of cognitive dysfunction following TBI, much of the existing data is preliminary, inconsistent, and not sufficiently conclusive in the overall efficacy and safety of the interventions. To date, many pharmacological clinical trials for TBI recovery have not produced consistently effective treatments capable of improving long-term cognitive outcomes, highlighting the complexity of TBI pathophysiology and the difficulty of developing targeted therapies.[3] This lack of confidence warrants the investigation into the potential efficacy and safety of NPIs.

While pharmacological interventions are effective at treating acute disruptions resulting from TBIs, the injury is dynamic with long-term consequences that often require continual management.[3] A TBI requires constant re-prescription of drugs, subsequently increasing the risk of adverse reactions and side effects with each new drug.[6] NPIs may offer certain advantages in TBI rehabilitation when compared to pharmacological interventions, including a lower risk of adverse effects and greater flexibility to adapt interventions to the evolving nature of recovery. They also present a considerably lower long-term economic burden on

healthcare systems compared to pharmacological therapies, which require constant research and development funding.

Methylphenidate, a pharmacological intervention that targets dopamine and norepinephrine pathways commonly impaired following TBI, has been shown to produce modest improvements in cognitive functioning in TBI patients (effect size d = 0.34, 95% CI: 0.12–0.56), particularly in processing speed and executive tasks.[4] However, as with many pharmacological interventions, these benefits should be considered alongside the potential for adverse effects. Although the following safety estimates were derived from a study of 71 children receiving methylphenidate for ADHD rather than patients with TBI, they are included to illustrate the broader tolerability profile of the medication across clinical populations. In that study, anorexia was reported in 74.3% of participants, irritability in 57.1%, and insomnia in 47.2%.[8] While these frequencies may not directly generalize to TBI populations, they highlight the importance of considering adverse effects when evaluating pharmacological approaches for cognitive recovery. Donepezil has demonstrated larger effect sizes for certain cognitive outcomes (d = 1.68), although this finding is based on a limited number of studies and requires further validation.[4] The following adverse effect estimates are likewise drawn from broader safety reporting rather than TBI-specific populations and are included to contextualize the medication's general tolerability profile. More than 10% of users experience nausea, headache, and diarrhea, while 1–10% experience insomnia, tremor, epigastric pain, hypertension, atrial fibrillation, eczema, nocturia, anorexia, hallucination, cataracts, and arthritis.[9]

It is important to note that many of the methodological limitations used to critique pharmacological studies are also found in non-pharmacological research and should be considered with equal weight. Across the field of TBI research, there remains a limited number of rigorous double-blind clinical trials, and the absence of validated biomarkers makes it difficult to objectively determine whether interventions are truly effective in human populations. In addition, the substantial heterogeneity of TBI injuries and patient characteristics complicates the interpretation of treatment outcomes and requires very large sample sizes to detect meaningful effects. The clinical TBI field is currently undergoing a reassessment of traditional mild, moderate, and severe classifications in an effort to better classify patient populations and improve the design and feasibility of clinical trials. These challenges increase the cost and complexity of conducting high-quality studies and apply to both therapies. While limitations in pharmacological trials are often scrutinized more heavily due to regulatory requirements and potential side effects, the methodological burdens are shared across all intervention types and reflect broader challenges within TBI research rather than weaknesses specific to any single treatment approach.

2. Virtual Reality as an Effective Tool for Cognitive Training, Spatial Orientation, and Balance

Virtual Reality (VR) has emerged as a promising NPI for TBI due to its capacity for task specificity, adaptability, and immersive cognitive engagement. VR is a technology that uses computer-generated simulations to create immersive, interactive environments. There are various iterations of VR targeting specific TBI-related cognitive dysfunctions, including executive dysfunction and balance. The customizability of VR opens a completely new field that requires rigorous investigation to determine the possible benefits and side effects of its iterations.

2.1 Task-Based VR Rehabilitation

A pilot controlled clinical study by De Luca et al. (2023) suggests that task-based VR has the potential to improve visuo-executive abilities, coping

strategies, and mood in TBI patients.[10] Participants were seated in front of a virtual device and performed various tasks aided by a therapist. Patients completed a VR rehabilitation system program of interactive visual exercises that targeted executive skills, attention, and problem-solving. The authors found that task-based VR rehabilitation may be a more efficient and cost-effective approach to improve coping strategies and mood in TBI patients than the current non-pharmacological treatment standard of rehabilitation. The current standard of care for a non-pharmacological task-based model is based on a face-to-face approach between a therapist and the patient using traditional materials such as paper and pencil tools. The task-based VR model is an expansion of this, offering greater adaptability and enjoyment that may be more effective at improving cognitive deficits following TBI.

2.2 Virtual Reality Orientation Therapy (VRot)

Similarly, an exploratory clinical study by De Luca et al. (2024) suggests that virtual reality orientation therapy (VRot) may optimize cognitive and behavioral functioning post-severe acquired brain injury.[11] The authors found that VRot may be a more efficient way of using simulated situations to reduce depressive symptoms and improve the reality orientation deficit caused by TBIs than standard reality orientation therapy (ROT). The current non-pharmacological model of ROT involves a classroom teaching approach or a guided household chore approach. The VRot used in this study used virtual scenarios that simulate real-world situations to repeat time, place, and person orientation with meaningful stimuli to help the patient develop a better understanding of their environment and gain a sense of control. The patients were presented with scenarios, such as driving, shopping at the supermarket, and household chores to perform on their own with virtual guidance. The authors found statistically significant improvements in cognitive function and depression in the subjects, larger improvements than those in the standard ROT group.

VRot therapy, like the task-based VR, offers greater adaptability and comfort for patients since a wide variety of simulations can be generated without having to travel anywhere and avoids the fear of possible consequences for choosing certain responses to social interactions. The data also suggests that this virtual extension may be more effective at improving spatial orientation than standard conventions. These two studies highlight the possible benefits of transforming current NPIs into virtual iterations.

2.3 VR Combined with Exercise

Another development in the VR field involves combining VR interventions with other NPIs. A randomized control pilot trial by Tefertiller et al. (2022) suggests that coupling VR rehabilitation with treadmill training may be a safer and more effective way to treat balance and endurance deficits post-TBI than treadmill training or stationary training on their own.[12] The patients in the VR-coupled treadmill training group interacted with virtual feedback games while walking at various paces on a treadmill. The authors found that no patients experienced serious adverse events and that the VR coupled with the treadmill group showed significantly greater improvements in balance and mobility than the solely VR and solely treadmill groups. While the sample size was small, the preliminary data highlights the possible benefits of combining a virtual reality model with other TBI-related non-pharmacological models.

2.4 Synthesis of the Literature

These three studies highlight the potential advantages of VR-based interventions over existing non-pharmacological rehabilitation approaches by increasing adaptability and patient engagement. Both the task-based VR and the VRot studies suggest that virtual extensions of the current standard of care outperform their traditional

counterparts in treating specific cognitive dysfunctions. Rather than requiring the invention of a new type of treatment framework, VR seems to enhance the current methods of treating cognitive dysfunctions and can potentially be used to build upon the framework already established to treat TBIs. This new enhancement allows researchers to further individualize treatments, repeat intervention exposure, and receive real-time feedback. The preliminary data from the coupled study proposes investigation into combining VR-focused rehabilitations with other low-risk NPIs, such as exercise, diet changes, and hyperbaric oxygen therapy, to evolve the standard of care for TBIs to minimize risks and increase adaptability and efficacy.

Some limitations to consider across these studies include small sample sizes and pilot or exploratory designs, which limit statistical power, generalizability to different TBI populations, and ability to draw definitive conclusions about efficacy. In the VRot study, the inclusion of severe acquired brain injury patients rather than exclusively TBI participants restricts the application of the conclusions to TBI-related deficits. For the VR combined with exercise study, it is important to consider its design as a pilot randomized controlled trial to determine whether the intervention was practical and safe to implement, rather than to determine its effectiveness. Additionally, its focus on balance and mobility outcomes restricts making broader conclusions about neurocognitive recovery. Short follow-up periods across studies also make it unclear whether observed improvements in executive function, mood, balance, and spatial orientation were sustained over time. These limitations suggest that while VR-based interventions demonstrate potential, the current evidence is preliminary and should be interpreted cautiously until further validated by larger, standardized trials.

Directly comparing the studies is challenging due to the differing outcome measures, iterations of VR, and cognitive dysfunctions targeted. Further research within each of these iterations should investigate the true efficacy of each intervention. Nonetheless, there is a promising pattern that suggests VR can improve and evolve the current standard of care for TBIs.

3. Hyperbaric Oxygen Therapy as a Neurorehabilitation Tool for Cognitive Function

Hyperbaric Oxygen Therapy (HBOT) is a treatment in which a patient is placed within a pressurized chamber, allowing the blood to carry more oxygen throughout the body, improving and accelerating recovery.

3.1 Clinical Evidence for Functional and Quality-of-Life Outcomes

A prospective clinical intervention study by Ablin et al. (2023) evaluated the effects of standard HBOT procedures as TBI treatments by having participants breathe 100% oxygen through a mask.[13] The study suggested that HBOT enhances activation in the frontal and temporal lobes, corresponding with improvements in pain control, emotional regulation, and cognitive function. Patients in the HBOT group reported greater satisfaction and improvements in quality-of-life measures compared with participants receiving pharmacological treatment (Pregabalin or Duloxetine). This was supported by significant increases in SF-36 Health Survey scores, a validated measure of health-related quality of life, where higher scores indicate better functioning, particularly in the vitality (energy), social functioning, and pain domains. While HBOT demonstrates promising clinical outcomes, comparisons with other NPIs remain limited as variability in protocols and outcome make it difficult. The evidence suggests that HBOT represents one of many potentially effective NPIs, and future research should explore its combined use with other NPIs to evaluate potential synergistic effects.

3.2 Mechanistic Evidence from Preclinical Models

A study conducted by Sakas et al. (2023) further supports HBOT's neurological recovery abilities.[14] They found that HBOT administered early in disease progression lessens the effects of the secondary injury cascade of metabolic dysfunction following TBI using a rodent-model. At a cellular level, increasing oxygen delivery may help injured neurons restore energy production. Post-TBI, mitochondrial dysfunction limits the amount of ATP made, leaving neurons without an energy source and more vulnerable to apoptosis. By improving oxygen availability, HBOT may support ATP-formation which stabilizes cellular energy balance thereby reducing secondary cell death. This neuroprotection also preserves mitochondrial function through increased oxygenation of the bloodstream.[14]

Sakas et al. (2023) also suggest that HBOT can decrease oxidative stress, reduce inflammatory signaling, and promote angiogenesis, potentially improving blood flow to damaged brain tissue. However, most of this mechanistic evidence comes from animal models, where oxygen dosing and timing are tightly controlled. While human studies have demonstrated improvements in cerebral blood flow, direct confirmation of the exact cellular mechanisms in the preclinical models in humans is limited.[13] Therefore, although these pathways work in theory, further translational research is needed.

Additionally, the protective mechanisms of reducing glial proliferation and apoptosis improve cognitive and motor recovery while also addressing the metabolic dysfunction issue, for which pharmacological interventions have shown inconsistent results.[4] These findings build upon those of Ablin et al. (2023) by confirming HBOT's potential benefits of improving cognitive function and cognition-linked metabolic processes.[13]

3.3 Clinical Evidence for Functional and Quality-of-Life Outcomes

These studies collectively demonstrate HBOT's potential as a treatment for TBI. Clinical findings suggest improvements in quality of life, including social and emotional functioning, while preclinical rodent studies provide mechanistic evidence that HBOT may reduce secondary brain damage and neuronal apoptosis following injury.[13,14] Additional clinical evidence further supports these findings.

One study conducted by Chen et al. (2022) examined whether HBOT could reduce hematoma volume in patients with TBI.[15] Differences in recovery were measured using Glasgow Coma Scale (GCS) scores and the Coma Recovery Scale–Revised (CRS-R). Consistent with the previous studies mentioned, the findings suggest that HBOT is an effective treatment to improve consciousness, cognitive function, and prognosis. As HBOT reduced hematoma volume, patients showed improved patterns of electrical brain activity on an electroencephalogram (EEG).[15] Hematomas can increase pressure in the cranium, compressing brain tissue and disrupting the neural signaling necessary for cognition and consciousness. By reducing the volume of hematomas, HBOT relieves this pressure buildup in the brain, facilitating blood flow and oxygen delivery to the brain, which is associated with neural recovery and improved EEG activity, as shown by the patients in the study.[12] Compared to Ablin et al. (2023), whose results depend on symptoms, this study demonstrates physiological benefits.[13] The above studies collectively demonstrate HBOT's versatility and treatment range as a NPI. These findings suggest that HBOT may influence aspects of TBI recovery, such as oxygenation and metabolic function.

Some limitations should be considered when interpreting these findings. In Ablin et al.'s (2023) study, many of the improvements were measured

using patient-reported outcomes and quality-of-life scales, which may be influenced by patients' expectations about the treatment rather than reflecting objective neurological improvement.[13] The sample size was also limited, which makes it difficult to generalize the results to the broader TBI population. In the preclinical study by Sakas et al. (2023), the use of a rat model limits translation of the results to humans, as recovery, brain function, and metabolic rates differ across species.[14] Additionally, laboratory conditions cannot fully replicate the complexity and variability of TBI cases in real life. In Chen et al.'s (2022) study, HBOT was administered alongside standard medical treatment rather than as a standalone intervention.[13-15] Therefore, although reductions in hematoma volume and improvements in GCS and CRS-R scores suggest additional physiological benefit, the multifactorial nature of TBI recovery makes it difficult to attribute these outcomes only to HBOT. Across studies, differences in oxygen dosage, treatment duration, and timing of intervention also make direct comparison challenging. Ultimately, while HBOT shows promising potential, the current evidence remains preliminary and should be regarded with caution until supported by larger clinical trials.

4. Nutritional Therapies as a Metabolic Enhancement Tool

A randomized controlled study by Yang et al. (2023) examined the effects of intermittent fasting (IF) in mice on the activation of the ferroptosis pathway, a type of programmed cell death that contributes to neuronal loss following TBI, and related outcomes.[16] IF is a dietary pattern that alternates between food consumption and absence from consumption. The major finding was that a 1-month period of IF in mice led to the alleviation of TBI-induced ferroptosis. This potentially aids cognitive repair by increasing metabolically active cells. The researchers reported that IF reduced markers of ferroptosis. By limiting ferroptotic signaling, more neurons survived and metabolism stabilized. The study also included behavioral cognitive testing, such as spatial learning and memory assessments, which demonstrated improved performance in mice undergoing intermittent fasting compared to controls. An important finding to note is that IF must be practiced for a long time to be effective. In this study, shorter periods of IF (<1 mo.) resulted in no significant changes in metabolic activity.

4.1 Intermittent Fasting and Neuroprotection

A review article by Finnegan and colleagues (2022) assessed the effects of nutrition on TBI recovery.[17] TBI exists along a spectrum of severity, including mild, moderate, and severe classifications based on neurological findings. Mild TBI encompasses the majority of cases and is characterized by subtle yet persistent cognitive and emotional deficits, despite limited structural abnormalities in the brain on imaging. While moderate and severe TBIs frequently require immediate, life-saving intervention right after the injury and intensive pharmacological management, mTBI recovery often relies more heavily on rehabilitation-based approaches that target neuroplasticity, metabolic repair, and functional reintegration. Therefore, mTBI provides a relevant framework to evaluate rehabilitative NPIs, which may have the greatest long-term impact on cognitive restoration. Post-mTBI (mild TBI), metabolic demand increases as the body prioritizes repair, resulting in greater nutritional requirements. An observational study analyzed in this review gave post-mTBI patients dietary journals to track how much food they were consuming in regards to calorie and protein intake. With the dietary journals, the researchers found that those who consumed the average dietary intake of 2,232 calories and 121.3 g/day, which served as a benchmark for evaluating patients' nutritional status. Patients whose intake

was near or met this benchmark were discharged earlier than those whose intake fell below these levels. This suggests that ensuring nutrition levels are met may improve recovery time from acute mTBI, as adequate calorie and protein intake are crucial in supplying the body with sufficient nutrients to facilitate recovery.

4.2 Synthesis of the Literature

The above findings suggest that nutrition is an important factor in TBI recovery. In fact, it may even be a more accessible intervention than some of the other discussed NPIs. For example, HBOT often requires repeated treatment sessions in specialized facilities, contributing to a treatment burden of travelling and time.[18] A recent Medicare cost analysis estimated that a standard course of 40 HBOT sessions cost approximately USD $23,834 per patient in 2022, with each session averaging $595.86.[18] In contrast, nutritional interventions can generally be incorporated into existing routines without requiring specialized infrastructure. However, other treatments may be more promising in terms of timeliness, as dietary modifications do not always result in drastic enough improvements necessary for recovery. It's important to note that direct cost-effectiveness comparisons between nutritional interventions and other TBI treatments have not yet been found.

Despite these promising findings, limitations should be acknowledged. Yang et al.'s intermittent fasting study was conducted on mice, which limits translation to human TBI populations, as metabolic processes, recovery timelines, and dietary needs differ between species. Additionally, the optimal duration, timing, and implementation of fasting protocols in clinical TBI populations remain unclear.[19] The observational study assessing calorie and protein intake in post-mTBI patients also relied on self-reported dietary journals, so it is difficult to determine whether earlier discharge was directly caused by nutritional intake or other factors. It is possible that patients with milder injuries felt better and were therefore able to eat more, that healthier individuals had stronger appetites, or that patients recovering more quickly consumed greater amounts of food. Furthermore, the absence of standardized dietary protocols limits the ability to form concrete clinical recommendations. Ultimately, while nutritional strategies show theoretical promise, the current evidence requires more human trials with clearer guidelines.

5. Exercise Interventions as a Tool for the Optimization of Surviving Cognitive Neuronal Networks

A preclinical study by Gomez-Porcuna and colleagues (2024) found that aerobic exercise (AE) using a motorized running wheel could improve cognitive recovery following TBI in rats.[20] AE is physical activity that uses large muscle groups in a consistent manner and relies on oxygen to make energy over an extended period of time. The target speeds were chosen as 8 m/min for TBI-8 (low intensity), 12 m/min for TBI-12 (moderate intensity), and 16 m/min for TBI-16 (high intensity). The researchers measured hippocampal volume, surviving neuron amounts, and neurogenesis after rat euthanization.

Short and long-term memory improvements in the object recognition test were observed across all exercise groups compared to TBI sedentary rats (TBI-sed); however, they did not reach baseline levels. This suggests that while exercise may support cognitive recovery following TBI, exercise alone may not be enough to restore function. One possible explanation is that adequate nutrition supplies the energy necessary to support the physical demands of exercise and promote recovery in surviving neural networks. This points to the potential value of combining interventions that target different aspects of recovery. Supporting this broader idea, Finnegan et al. (2022) found that patients who met nutritional

benchmarks during acute mTBI recovery were discharged earlier, suggesting that recovery may benefit from addressing factors like energy availability rather than relying on a single intervention.[17] However, because Finnegan et al. studied humans while these exercise findings were observed in rats, this comparison should be interpreted with caution.

One potential explanation for the combined effects of AE and IF on cognitive recovery is that IF has been shown to produce metabolic improvements by preventing neuronal cell death after four weeks.[17] By limiting neuronal loss, IF could preserve a greater number of surviving neural networks post-TBI. In contrast, AE improves the function of existing neural networks, as demonstrated in this preclinical study by memory improvements observed after four weeks of exercise. Therefore, if IF helps preserve neurons while AE enhances the function of surviving networks, the combined effects of these interventions may lead to greater improvements in cognitive recovery. Although hippocampal volume loss and neuronal death were not fully affected by exercise, both sexes in the exercise groups, independent of intensity, showed recovery in memory retention that came with enhanced neurogenesis and reduced microglial activation.

Nonetheless, several limitations affect how these findings are interpreted. This study was conducted in a rodent model, which limits direct translation to human TBI populations. The researchers also ensured exercise intensity and duration were strictly followed by the rodents, whereas human patients may vary widely in motivation, fatigue tolerance, and physical capacity.

5.1 Synaptic Plasticity and Neurotransmitter Regulation

Another preclinical study by Bonsale et al. (2023) found that long-term treadmill exercise could reverse cognitive and emotional injury in male mice post-TBI.[21] The TBI exercise (TBI-ex) mice ran on the treadmill for 8 weeks for 20 minutes every day at 5 min at 8 m/min, 10 min at 12 m/min, then 5 min at 5 m/min (cool-down). The study tested behavior (as a measure of cognitive recovery) through an open field, depression, and aggression test, as well as neurotransmitters and synaptic plasticity. Synaptic plasticity was evaluated through long-term potentiation (LTP). Beyond mechanistic findings supporting AE as a non-pharmacological intervention, this study demonstrated that AE directly improves emotional regulation. Cognitive recovery post-TBI is often limited by emotional barriers beyond memory and attention, including anxiety or depression that often interferes with learning and motivation to continue an intervention.

Behaviorally, researchers found the TBI-ex had a much higher exploration time, suggesting lower anxiety, lower fighting time, and a lower immobility time than TBI-sed. This suggests that AE could be a viable non-pharmacological intervention for promoting emotional regulation and social cohesion by reducing anxiety, aggression, and depression. In the hippocampus and nucleus accumbens, there were elevated levels of glutamate and gamma-aminobutyric acid (GABA) post-TBI, disrupting the balance between excitatory and inhibitory signaling and contributing to neuronal imbalance. The hippocampus contributes to learning and memory processes, while the nucleus accumbens is involved in reward processing, motivation, and emotional behavior.[22] Disruptions in neurotransmitter signaling within these regions post-TBI contribute to cognitive impairment and emotional dysregulation. TBI-ex had much lower levels of both compared to TBI-sed. Many pharmacological interventions have shown inconsistent results on neurotransmitter balance.[23] Conversely, these findings suggest that AE improves cognition post-TBI by restoring different types of neurotransmitters at the same time, fixing the damage itself in areas of the brain that control

memory, emotion, and motivation. Compared to TBI-sed, TBI-ex also had stronger synaptic plasticity. Because LTP is the mechanism behind memory and learning, the TBI-ex findings suggest that exercise may support cognitive recovery by enhancing synaptic plasticity and strengthening communication within surviving neural networks.[24] However, LTP is likely only one of several mechanisms contributing to these improvements.

These findings also suggest that maximizing the benefits of AE may require adjusting exercise intensity based on sex, supporting the idea that personalized intervention plans may be more effective than standardized treatment approaches.[20] The finding that AE repairs communication in neural networks also complements Yang et al.'s (2023) finding that IF suppresses ferroptosis, by suggesting that cognitive recovery needs to both protect surviving neural circuits and prevent further neuronal death.[16] Gómez-Porcuna et al. (2024) demonstrated what outcomes improved after AE, while Bonsale et al. (2023) offer mechanisms behind these improvements.[20,21] These studies support the idea that an effective intervention does not need to replace the damaged neural tissue, but rather improve upon the efficiency and plasticity of surviving neural networks to restore cognition.

An important limitation to consider here, as with the other preclinical models, is that the use of male mice limits generalizability across sexes and to human populations.

5.2 Clinical Evidence

Clinically, López et al. (2024) investigated the effect of exercise on memory-like executive function.[25,20] Participants completed supervised AE three times per week and were evaluated for executive function using a test for cognitive flexibility, attention, and memory. The exercises were personalized based on their physical capability and included treadmill walking, stationary cycling, and use of a motorized leg trainer. They found that 20-week AE programs could help improve executive function in six participants with severe chronic TBI. Although Gómez-Porcuna et al.'s (2024) findings came from a rat model during the acute phase of TBI recovery, López et al.'s clinical study observed benefits of AE in patients during the chronic phase.[20,25] While these studies cannot be directly compared, they suggest that exercise may have a wide intervention period. This also connects to Bonsale et al.'s (2023) finding that the TBI-ex group had less anxiety, aggression, and depression than the TBI-ex group because executive function depends on emotional regulation.[21] By first improving emotional regulation, AE removes barriers to cognitive improvement, which could facilitate recovery of executive functions.[21,25] The personalized design with the motorized leg trainer also highlights one of NPIs' greatest strengths compared to pharmacological interventions, which are typically applied to every patient equally. There are few pharmacological interventions available for treating cognitive recovery post-TBI, and while doctors can change dosages and medication timing, the medications themselves are standardized. The drugs are made to target broad areas rather than certain patterns or recovery trajectories specific to the patient. As a result, pharmacological interventions follow a trial-and-error approach that fail to address the root neurological issues.[26]

These gains in the chronic phase not only support AE as an effective non-pharmacological intervention, but also challenge the assumption that cognitive recovery plateaus at the chronic level and demonstrate that targeting behavior using exercise as an intervention can create measurable cognitive improvement. Researchers also found that the AE "spilled over" into participants' daily behavior, which is especially valuable in TBI recovery, as consistency is a necessity for meaningful improvement. This is in contrast with pharmacological interventions, which focus on symptom management rather than

behavioral changes, and have limited use in the chronic phase.[27]

These findings should be interpreted with caution due to limitations. The clinical study included only six participants, limiting generalizability. The absence of a large randomized control group makes it difficult to isolate aerobic exercise as the sole contributor to improvements in executive function, as gains may have been influenced by therapist interaction or healthier habits.

6. Conclusion

Overall, NPIs demonstrate potential for improving cognitive function impaired by TBIs. Due to the long-term, often worsening, effects of TBI, a non-pharmacological treatment plan can mitigate the side effects and risks of current pharmacological treatments. The preliminary trials of NPIs have shown significant improvement in recovery post mild-TBI, and clinical studies aim to increase applicability for more severe TBI cases. VR-based interventions demonstrated improvements across several domains relevant to TBI recovery, including cognitive functioning, emotional regulation, and physical rehabilitation outcomes. Beyond this, VR's immersive and adaptable design may enhance patient engagement as it is often enjoyable and allows therapies to be tailored to individual recovery needs. Similarly, HBOT showed promise as a NPI by targeting physiological mechanisms associated with secondary injury following TBI. Rather than focusing solely on symptom management, HBOT may influence underlying biological processes such as cerebral oxygenation, metabolic dysfunction, and neuronal survival. However, HBOT is expensive and requires extensive training and special equipment, limiting accessibility depending on a patient's location and financial circumstances. A focus on nutrition improved the speed of recovery as well as pain from the body trying to recover post-TBI. However, dietary recommendations are subjective, making this a difficult intervention to prescribe compared to other NPIs. Exercise helped improve memory, learning, and emotional regulation; however, it is important to acknowledge the differences between mice and human models and how the efficacy of these treatments can change between the two. Exercise is one of the most accessible NPIs, as it can often be done in the home and be personalized to the patient.

However, current research on NPIs remains limited due to studies with small sample sizes and misunderstood mechanisms of recovery. Future research should focus on expanding sample sizes and standardizing outcome measures to make results more applicable to mild, moderate, and severe TBIs. It is also critical for researchers to work on coupling these approaches with existing pharmacological approaches to see how they may improve the standard of care and cognitive recovery post-TBI.

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About the Authors

Ayla Feng

Ayla Feng is a sophomore majoring in Health Care Management and Policy in the School of Health. She is interested in researching chronic disease and women's health, and she hopes to work towards addressing inequities in healthcare that arise from social and economic barriers to care.

Nyla Wells

Nyla Wells is a sophomore in the School of Health majoring in Health Care Management and Policy. She is passionate about addressing health disparities and improving health outcomes within underserved communities.

Cecilia Hanuch

Cecilia Hanuch is an undergraduate student ('27) in the School of Health, majoring in Health Care Management and Policy on the pre-health track. She is interested in improving women's health and reducing implicit bias through equalitarian health policies. She is a diver on the Swim and Dive team and hopes to pursue dentistry.

Benjamin Cohen

Benjamin Cohen is a junior in the Georgetown College of Arts & Sciences, majoring in Neurobiology with minors in Chemistry and Bioethics. He studies an axon guidance gene and its role in neurodevelopmental disorders in the Coate Lab, and gets his dendrites tickled by independent research on clinical interventions for post-sepsis impairment in the blood-brain barrier and the neuroethics of head transplantation. Drawn to neurosurgery for its mathematical precision to restore physical substance with the profoundly human task of safeguarding consciousness, Benjamin aspires to bridge research and life-altering outcomes at the patient's bedside.

About the Authors

Fritze Mayer

Fritze Mayer is a recent graduate of the Class of 2026 from the School of Health at Georgetown University, where she studied Global Health and Statistics. Her academic interests center on the intersection of health and nutrition within the broader social determinants of population health, with a particular curiosity for the role food and diet play across cultural contexts.

Eric Feichthaler

Eric Feichthaler is an undergraduate (CAS '27) studying environmental biology. He has worked in the Wimp Lab for two years and spent a semester in Tanzania where he completed his own research. He is especially interested in megafauna, which he has learned about during his travels to all 63 of America's national parks.

Neha Nimma

Neha Nimma is a sophomore at Georgetown University's School of Health majoring in Human Science on the pre-medical track. She is passionate about endocrine and cancer research and will be working in Dr. Shepard's lab to identify and study orphan receptors in the human body. In addition to her academic interests, she is actively involved in diabetes awareness initiatives and helps lead volunteer pop-up clinics throughout the D.C. area.

Piper Levin

Piper Levin is a member of the Class of '29 at Georgetown University majoring in Human Science. Originally from Scituate, she is passionate about cancer research, with a particular interest in rare and aggressive tumors. Piper will be working on Dr. Merenstein's NOSES study next year, further exploring clinical research and its impact on patient care. She hopes to pursue a career as a surgeon and is dedicated to advancing treatment and outcomes for patients facing complex diseases.

About the Authors

Emily Ricigliano

Emily Ricigliano is a member of the class of 2029 and is majoring in Human Science in the School of Health on the pre-med track. She is passionate about exploring pediatric oncology and how to minimize fatal side effects while delivering the most effective treatment.

Sophia Shin

Sophia Shin is a sophomore at Georgetown University's School of Health, majoring in Human Science. Originally from Los Angeles, California, she is passionate about pursuing a career in the medical field and plan to continue exploring opportunities at the intersection of science and clinical care.

Leon Wang

Leon Wang is a sophomore at Georgetown University's School of Health, majoring in Human Science with a minor in Statistics. He is passionate about immunopeptidomics research and its potential to advance our understanding of disease at the molecular level. He plans to pursue a career in medicine.

Ahursa Maryam Ghalebi

Ahursa Maryam Ghalebi is an undergraduate student in the School of Health at Georgetown University ('29), majoring in Human Science and minoring in Spanish. She is passionate about learning languages and advancing neuroscience research. After graduation, she plans to pursue medical school.

About the Authors

Shruthy Thamizhavel

Shruthy Thamizhavel is a sophomore in the School of Health, majoring in Human Science on the pre-med track. She is interested in advocating for the use of non-pharmacological treatments in aiding TBI and stroke recovery.

Natalie Carousso

Natalie Carousso is a sophomore in the School of Health ('29), majoring in human science on the pre-med track. She is looking forward to expanding her research in neuroscience, specifically focusing on stroke recovery and TBI.

Viviana Méndez Vélez

Viviana Méndez Vélez is an undergraduate student in the School of Health ('29) majoring in Human Science on the pre-medical track. Born and raised in Puerto Rico, she is passionate about enhancing health outcomes and promoting health equity for the Latino population throughout the United States.

Acknowledgements

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