**Abstract**

This study adopts a dimensional and transdiagnostic framework, treating Orthorexia Nervosa (ON) as an ideologically driven eating pattern situated along a continuum of health-oriented behaviours. It acknowledges that ON is not yet defined as a discrete psychiatric disorder; thus, it seeks to map its biopsychological correlates with established eating and obsessive-compulsive conditions. The goal is not to validate ON as a discrete diagnosis but to identify transdiagnostic biopsychological patterns associated with high orthorexic tendencies, informing future classification debates without presuming categorical status. This project will examine ON across four key domains—psychological, immunological, neurophysiological, and microbiome.

ON is an emerging, under-recognized mental health condition characterized by a pathological obsession with consuming foods perceived as pure or healthy. Although growing clinical attention has differentiated ON from both Healthy Orthorexia (HO) and traditional eating disorders such as Anorexia Nervosa (AN) and Bulimia Nervosa (BN), its biological foundations remain largely unexplored. The proposed project aims to comprehensively characterize ON across psychological, immunological, gut microbiome, and neurophysiological domains and distinguish it from HO, AN, and healthy controls (CG). Due to the current lack of empirical data in ON, the gut microbiome and ERP measures will be treated as *exploratory, hypothesis-generating components*. While guided by findings from AN and OCD, these analyses are designed to inform future confirmatory studies rather than provide diagnostic or causal conclusions. A longitudinal assessment and data-driven integration of findings may be considered as time permits and based on data quality and feasibility. This project addresses a significant gap in current mental health research. It aims to generate critical foundations for early intervention strategies and public health policy in the global rise of wellness culture. This study will not presume or assert that Orthorexia Nervosa constitutes a discrete psychiatric or pathophysiological entity. Instead, biological data will be interpreted as markers of psychological or behavioral correlates, without implying causality, disease status, or nosological validity. Even if statistically significant differences are found, they will be treated as construct-related findings within a dimensional framework.

**Scientific Background**

Orthorexia Nervosa (ON) describes an emerging, under-recognized mental health condition characterized by a pathological obsession with consuming foods perceived as pure or healthy[1]. While Healthy Orthorexia (HO), an interest in nutritious eating, may support wellbeing and psychological resilience, Orthorexia Nervosa (ON leads to significant emotional distress, interpersonal difficulties, and medical complications [1, 2]. Despite growing recognition, the diagnostic status of ON remains highly contested. It is not currently included in major psychiatric classification systems (DSM-5, ICD-11), and there is no consensus on whether ON should be conceptualized as a standalone disorder, a subtype of Anorexia Nervosa (AN) or Obsessive-Compulsive Disorder (OCD), or a culturally constructed manifestation of extreme health-focused behaviour. This ambiguity raises critical theoretical and methodological concerns. The present proposal adopts an exploratory stance, seeking to clarify ON's biopsychological profile concerning established disorders. However, it must be acknowledged that the construct of ON may reflect a heterogeneous or ideologically shaped phenomenon rather than a nosologically discrete condition [1, 3, 4]. Addressing this knowledge gap is imperative, particularly in light of the global rise in wellness culture, which increasingly promotes dietary restriction and “clean eating” ideologies that may mask or catalyze disordered behaviours [5].

Furthermore, the study relies on the Teruel Orthorexia Scale (TOS) to define and classify ON and HO. While the TOS has shown promise and was recently validated in Hebrew by the PI’s group [6], its broader psychometric robustness, clinical applicability, and diagnostic validity are still under evaluation in the field. These limitations must be explicitly acknowledged when interpreting results, particularly when drawing nosological conclusions. Future work should incorporate critical and dimensional perspectives to avoid reifying a construct whose diagnostic boundaries remain fluid.

Psychologically, ON is characterized by extreme rigidity, perfectionism, anxiety, and obsessive-compulsive traits [1, 2, 7]. While these features resemble aspects of Anorexia Nervosa (AN) and Obsessive-Compulsive Disorder (OCD), ON is distinct in its motivational structure: individuals are driven not by weight loss or body image concerns, as in AN and Bulimia Nervosa (BN), but by a desire for health, purity, and moral virtue [8, 9]. However, despite these psychological distinctions, ON has yet to be evaluated systematically across biological domains known to play key roles in the development and maintenance of established eating disorders. The present research aims to fill this gap by comprehensively examining ON across four key biopsychological dimensions: immune biomarkers, gut microbiome composition, neurophysiological function, and psychological symptomatology.

*Immune Dysregulation in Eating Disorders*

A robust body of research demonstrates that eating disorders are associated with chronic low-grade inflammation. In particular, AN has been linked to elevated concentrations of pro-inflammatory cytokines, including interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-alpha (TNF-α) [10, 11]. These inflammatory markers are not merely by-products of malnutrition but have been shown to interact with psychological symptoms such as anxiety, fatigue, and cognitive rigidity [12, 13]. While it is hypothesized that ON may also involve immune dysregulation, potentially via stress-related inflammation, no studies have examined cytokine profiles in ON to date. This represents a crucial gap, particularly given the physiological stress and nutritional restriction observed in ON behaviours [14]. Exploring ON’s immunological signature will clarify whether it shares an inflammatory basis with AN or represents a milder, unique profile.

*Gut Microbiome Disruptions*

This component is hypothesis-generating. Taxonomic findings will be interpreted descriptively and not treated as diagnostic or mechanistic inferences. The gut-brain axis is a key mechanism linking dietary behaviours, immune signalling, and mental health. Individuals with AN consistently demonstrate reduced microbial diversity, altered ratios of Firmicutes to Bacteroidetes, decreased short-chain fatty acid-producing bacteria levels, and increased presence of pro-inflammatory taxa such as *Proteobacteria* and *Enterobacteriaceae* [15–17]. These alterations have been associated with increased intestinal permeability, systemic inflammation, and potential effects on appetite regulation and mood [18, 19]. Given ON’s rigid and often restrictive dietary patterns — including elimination of entire food groups and reliance on limited “safe” foods — similar microbiome shifts may be expected. However, the gut microbiome has not yet been studied in ON, and it remains unclear whether ON is associated with the same degree of dysbiosis or reflects distinct microbial patterns related to orthorexic eating.

*Neurophysiological Abnormalities (EEG/ERP) – exploratory component*

Neurophysiological studies using electroencephalography (EEG) have revealed that individuals with eating disorders, especially AN, exhibit altered patterns of resting-state brain activity and atypical event-related potentials (ERPs) in response to food- and emotion-related stimuli [20, 21]. ERP components such as the P300 (associated with attentional allocation and cognitive control), N200 (linked to conflict monitoring), and the Late Positive Potential (LPP, related to sustained emotional attention) are frequently disrupted [22, 23]. These abnormalities reflect broader deficits in executive functioning, affect regulation, and attentional flexibility, which mirror the rigid and perfectionistic thought patterns observed behaviourally. Given the strong psychological parallels between ON and AN, including heightened anxiety, obsessive traits, and cognitive inflexibility [1, 7, 8], it is plausible that ON may also exhibit similar EEG/ERP signatures. Yet, since no neurophysiological research has been conducted on ON, this component remains *exploratory* due to the absence of prior ERP studies in ON. ERP results will be interpreted cautiously to guide future research on attentional and emotional processes in ideologically driven eating behaviours.

As no ERP studies have been conducted on ON, neural markers will be selected based on well-established findings in related disorders such as AN and OCD [1, 8, 9, 24], which share key psychological traits with ON. The dedicated neurophysiology section below describes the ERP rationale and task design. ERP research in AN has demonstrated attenuated P300 amplitudes and altered N200 responses during food-related and emotionally salient tasks, reflecting deficits in attentional control and emotional processing [23, 25]. Similarly, OCD studies have revealed increased N200 negativity and shorter latencies for both N200 and P300 components, indicative of heightened conflict monitoring and stimulus sensitivity [26, 27]. These findings provide a conceptual rationale for targeting similar ERP components in ON. However, due to ON’s unique ideological and health-oriented motivational structure, the paradigms will be validated during the pilot phase, and task modifications will be implemented to ensure construct sensitivity and psychometric reliability.

**Conceptual Approach and Diagnostic Considerations**

We aim not to confirm ON as a clinical entity but to map its biopsychological correlates compared to known disorders. The study adopts a construct validity framework, recognizing ON’s fluidity and cultural embeddedness. This approach allows us to empirically investigate the construct without prematurely pathologizing health-oriented behaviours that may lie on a normative–pathologic continuum.

Given that ON is not formally recognized in the DSM-5 or ICD-11, the current study does not assume its status as a diagnostic entity. Instead, the research is guided by an exploratory and transdiagnostic perspective that treats ON as a working construct characterized by ideologically driven dietary rigidity and health-obsession. This approach allows the investigation of ON’s biological and psychological correlates without prematurely framing it as a psychiatric disorder. Findings may support, refine, or challenge current conceptualizations. However, this project explicitly avoids using the data to define ON as a clinical disorder. Instead, it aims to identify biologically and psychologically relevant patterns associated with orthorexic tendencies to inform, rather than determine, future diagnostic frameworks. This stance aligns with emerging calls to understand new health-related phenomena as behaviourally and culturally situated syndromes, rather than prematurely categorizing them as disorders without mechanistic evidence or cross-cultural validation.

**Integration and Innovation**

No published research has systematically examined psychological, immune, and neurophysiological profiles to define ON’s biological architecture. While the gut microbiome may play a role in ideologically restrictive eating, it will be treated as an exploratory, hypothesis-generating component due to its complexity and interpretive limitations. This is also true concerning a multimodal investigation of ON using these four domains in parallel. Such a framework aligns with recent calls in psychiatric research for transdiagnostic, biologically-informed approaches that can move beyond symptom descriptions to uncover mechanistic pathways and biomarkers.

Orthorexia AN shares several psychological and behavioral features, such as perfectionism, obsessive control over food intake, and elevated anxiety [2, 3, 24]. AN is thus a particularly informative comparison group for identifying overlapping and divergent biological profiles. In contrast, BN, while offering insights into the broader spectrum of disordered eating, is driven by different motivational and emotional processes (e.g., weight concerns and shame following binge episodes) and exhibits distinct biological patterns related to purging behaviours and binge cycles [28]. Thus, including AN and HO alongside healthy controls allows the proposed study to isolate ON-specific biological and psychological features and determine whether ON is best conceptualized as a distinct clinical entity or a variant within the eating disorder spectrum.

Given the increasing societal emphasis on “clean” eating, health optimization, and self-regulation [29, 30], ON may represent a culturally reinforced disorder with potentially widespread consequences. By incorporating cytokine profiling, microbiome sequencing, EEG/ERP measures, and psychometric evaluation in the same cohort, the proposed research offers a novel contribution to both clinical science and public health. This work will yield ON’s first evidence-based biological model, inform its classification and diagnosis, and guide early detection and intervention strategies.

Including four domains—psychological, immune, neural, and microbiome—reflects an integrative systems approach to ideologically driven eating behaviours. For example, inflammatory cytokines (IL-6, TNF-α) have been linked to cognitive rigidity and anxiety, core traits of ON. These traits may manifest as altered ERP responses (e.g., N200 or P300), indicating attentional and emotional dysregulation. Simultaneously, gut microbial profiles may reflect or even exacerbate this neuroinflammatory and cognitive-affective rigidity. By examining ON across these domains, the study can identify converging patterns that offer a multidimensional understanding of how ideological beliefs about purity may become biologically and psychologically entrenched.

**Research Objectives and Expected Significance**

*Research Objectives*

This study’s overa aimlore ON's biopsychological architecture by integrating psychological, immunological, gut microbiome, and neurophysiological data. Although ON has received growing attention, its status as a distinct psychiatric entity remains under debate. This study adopts a cautious, exploratory stance to identify whether ON exhibits unique patterns across key biological and psychological domains and how it compares to HO, AN, and non-disordered controls (CG).

*Primary Objective*

This study aims to investigate the core biopsychological features of ON by examining psychological, immunological, neurophysiological, and gut microbiome markers and comparing them with HO, AN, and CG.

*Specific Objectives*

1. **Psychological Profiling:** To identify cognitive, emotional, and behavioral traits—such as anxiety, perfectionism, rigidity, and obsessive-compulsive tendencies—that uniquely characterize ON, using validated psychometric instruments.
2. **Immunological Analysis:** To characterize ON-specific inflammatory profiles by measuring circulating cytokines (e.g., IL-6, IL-1β, TNF-α, IL-10) and comparing them across ON, AN, HO, and CG groups.
3. **Neurophysiological Assessment:** To examine resting-state EEG and event-related potential (ERP) responses to food-related and self-referential stimuli, identifying neural activity patterns that may distinguish ON from related conditions.

*Secondary (Exploratory) Objectives*

1. **Gut Microbiome Characterization (Exploratory):** To analyze ON participants’ gut microbial diversity and taxonomic composition, assess associations with restrictive or ideologically driven dietary patterns, and compare findings across diagnostic groups.
2. **Multimodal Integration:** If time and data quality permit, exploratory efforts will be made to integrate these findings into a preliminary model of ON’s biopsychological profile using traditional multivariate techniques (e.g., correlation matrices, linear regressions, PCA). This step is **deferred to Years 4–5** of the project. This postponement allows for sufficient data accumulation, quality assessment, and preliminary validation of primary analyses before engaging in high-dimensional exploratory modelling. Primary analyses will be prioritized to avoid inflation of false discovery rates, with correction procedures (e.g., FDR control) implemented.
3. **Temporal Stability (Longitudinal Follow-up):** To evaluate the 12-month stability of key psychological and biological markers in a subset of participants, assessing whether ON-related features represent enduring traits or state-dependent fluctuations. While the primary dataset is cross-sectional, the 12-month follow-up will provide preliminary insights into symptom and biomarker trajectories. However, we acknowledge that this limited follow-up in 30% of the sample cannot support strong causal claims. Rather, it is designed to identify potentially stable markers for future longitudinal or interventional studies. Findings will be used to generate hypot ON progression and recovery rather than confirm long-term etiological pathways.

An integrated analysis of psychological, immune, and neural data will allow us to move beyond symptom-level comparisons and identify latent ON profiles with theoretical implications for classification models and clinical differentiation.

*Expected Significance*

1. **Diagnostic Precision and Nosological Clarification**: This research addresses the current lack of formal diagnostic criteria for ON. By identifying its distinct or overlapping biological and psychological features, the study will clarify whether ON represents a standalone clinical entity, a subtype of existing eating disorders, or a maladaptive variant of normative health behaviour. Such data will inform future nosological debates, including potential inclusion of ON in the DSM or ICD frameworks.
2. **Identification of Biomarkers for Early Detection and Risk Stratification**: Integrating immune, microbial, and neurophysiological measures offers a pathway to developing objective biomarkers for ON. These may facilitate early detection in at-risk individuals, particularly those initially exhibiting non-pathological health-oriented behaviours, and distinguish ON from both HO and traditional eating disorders. This is particularly relevant in the context of increasingly blurred boundaries between wellness culture and disordered eating.
3. **Advancement of Gut-Brain-Axis and Neuroinflammation Models in Mental Health**: By connecting gut microbiota, immune markers, and brain-based EEG/ERP indices, this study will contribute to the broader scientific understanding of how diet, inflammation, and neural processing interact in the context of mental health. Findings could refine or expand current models of the gut-brain axis in eating disorders and obsessive-compulsive spectrum conditions.
4. **Development of Targeted Prevention and Intervention Strategies**: Understanding ON’s biopsychological profile will support the design of targeted interventions aimed at improving emotional regulation, reducing dietary rigidity, and modulating physiological markers (e.g., inflammation, microbiome composition). It will also inform prevention strategies for individuals at risk of transitioning from healthy dietary interests to pathological patterns.
5. **Public Health Relevance Amidst Global Wellness Trends**: With the global increase in wellness culture, plant-based dieting, and online “clean eating” communities, ON represents an emerging public health concern. This research responds to a pressing need for scientific grounding in discussions of wellness-related psychopathology and offers policy-relevant insights for health education, clinical guidelines, and community-based interventions.
6. **Methodological Innovation and Transdiagnostic Discovery**: The proposed study is methodologically innovative, integrating multimodal biological and psychological data in a unified framework.

Findings will be synthesized into an online screening brief and disseminated to Israeli clinical dietitians, school counsellors, and primary care networks. The PI will coordinate with the Ministry of Health’s public education division to disseminate findings through clinical workshops, briefings, and academic publications targeting dietitians, therapists, and primary care professionals.

**Detailed Description of the Proposed Research**

*Working Hypotheses*

The proposed project aims to decode the biopsychological underpinnings of ON, a condition that remains biologically uncharted despite increasing clinical attention. We hypothesize that ON, compared to HO, AN, and CG, we hypothesize that ON will exhibit unique profiles across psychological, immunological, microbiome, and neurophysiological domains.

*Primary Hypothesis*

Individuals identified with high orthorexic tendencies (as operationalized by the TOS) will exhibit psychological, immune, gut microbiome, and neural profiles that may differ from those observed in HO, AN, and CG. These differences will be examined to explore whether ON reflects a distinct biopsychological profile or shares transdiagnostic features with other eating- and obsession-related conditions.

*Secondary Hypotheses*

1. ON and AN groups will exhibit significantly elevated psychological rigidity, anxiety, perfectionism, and obsessive-compulsive traits compared to HO and CG.
2. Pro-inflammatory cytokines (IL-6, IL-1β, TNF-α) will be significantly higher in ON than in HO and CG, but lower than in AN.
3. Resting-state EEG and ERP responses (particularly N200, P300, and LPP) to food-related and self-referential stimuli will be altered in ON compared to HO and CG, reflecting disruptions in attentional control, emotion regulation, and conflict monitoring.

*Exploratory hypothesis*

1. ON may be associated with altered microbial diversity and increased pro-inflammatory taxa (e.g., Proteobacteria). These analyses will be treated as hypothesis-generating and conducted only if data quality and statistical power permit.

**Research Design**

This **cross-sectional study** uses a focused multimodal design, integrating psychological assessment, EEG/ERP neurophysiology, and immunological profiling as core domains. A **12-month longitudinal follow-up** will be conducted in a subsample to assess temporal stability in key psychological and biological markers. A priori power analysis was conducted using **G\*Power 3.1.9.6** to determine the minimum sample size required for detecting medium effect sizes across between-group comparisons in the primary domains of interest (psychological measures, EEG/ERP, and immunological biomarkers). A **one-way ANOVA** comparing four independent groups (ON, HO, AN, CG) was specified for the primary analysis, with an anticipated **medium effect size (f = 0.25)**, **alpha = .05**, and **power = 0.90**. This yielded a required total sample size of **approximately 280 participants**. To account for potential attrition, data loss (e.g., EEG artifacts, unusable biological samples), and the inclusion of covariates in secondary analyses (e.g., age, BMI, menstrual phase), the final planned sample was increased to **n = 300** (ON = 80, HO = 80, AN = 60, CG = 80). This allocation provides sufficient power to detect meaningful group differences in core psychological and biological outcomes. For the 12-month longitudinal follow-up (n ≈ 90), **linear mixed-effects models** will be employed, which are robust to missing data and suitable for repeated-measures designs. Prior power simulations based on similar longitudinal EEG and cytokine studies indicate that this subsample size allows detection of within-person changes with medium effect sizes (Cohen’s *d* ≥ 0.4) with power > .80, assuming standard attrition rates.

Thus, the study will recruit **300 participants** across four groups as follows:

* **ON Group (n=80):** Individuals meeting operational criteria for ON (via the Teruel Orthorexia Scale, TOS).
* **HO Group (n=80):** Individuals highly interested in healthy eating without pathological impairment.
* **AN Group (n=60):** Individuals meeting DSM-5 criteria for Anorexia Nervosa. The smaller sample reflects both recruitment feasibility and the project's exploratory scope.
* **Control Group (CG; n=80):** Non-eating-disordered healthy controls, age- and sex-matched.

Participants will complete psychological assessments and provide blood (cytokines), stool (microbiome), and EEG data. A subset (~30%) will undergo follow-up testing after 12 months.

**Methods**

*Ethical Considerations*

The study will be conducted under the Declaration of Helsinki and the ethical guidelines for human research established by the Israeli Ministry of Health. Ethical approval will be obtained from the Institutional Review Board (IRB) at Tel-Hai Academic College before the commencement of participant recruitment. All participants will provide written informed consent after receiving detailed information about the procedures, risks, benefits, and their right to withdraw from the study at any stage without penalty.

Given the sensitive and potentially invasive nature of specific components of this research, the following risk mitigation strategies will be implemented:

* **Emotional Distress During ERP Tasks**: ERP paradigms involving food-related and self-referential stimuli may elicit emotional discomfort, particularly among participants with a history of disordered eating. All tasks will be piloted and designed with validated stimuli that minimize distress while capturing relevant cognitive-affective processes. Participants will be fully briefed and may skip any stimuli or withdraw from the task without penalty.
* **Infection Risk from Blood Draws**: A licensed nurse trained in sterile technique will perform all blood sampling. Procedures will follow national biosafety protocols, and participants will be monitored for adverse reactions. All equipment (needles, tubes, gloves) will be single-use and disposed of according to clinical waste regulations.
* **Privacy and Dignity in Stool Sample Collection**: Participants will collect stool samples in the privacy of their own homes using sterile, pre-packaged kits with detailed written and visual instructions. They will return samples in sealed containers using secure collection boxes at designated locations.
* **Participant Withdrawal and Vulnerability Protections**: Special attention will be given to participants with clinical diagnoses (e.g., AN), who may be psychologically or physically vulnerable. These individuals will be monitored throughout participation, with regular check-ins and optional breaks. A clinical psychologist will be available for consultation and referral if distress arises. All participants will be reminded of their right to withdraw without impacting any treatment or services they receive.
* **Data Confidentiality**: All data will be anonymized using unique participant codes. Identifying information will be stored separately from research data on password-protected, encrypted systems accessible only to the core research team. Data sharing and publication will only be conducted at the group level, with no personally identifiable results.

All participants with a clinical diagnosis will be cleared for participation by their attending psychiatrist or physician. A licensed clinical psychologist will be available on-site for support. Emotional distress during food tasks will be closely monitored, with opt-out options and immediate withdrawal allowed. These ethical safeguards are designed to balance the scientific value of this multimodal investigation with a strong commitment to participant safety, autonomy, and dignity.

*Participants*

Participants will be recruited via eating disorder clinics, academic campuses, social media platforms, and community networks. Inclusion criteria include age 18–45, fluent Hebrew, and no neurological or major systemic illness. Exclusion criteria include active psychosis, recent antibiotic use (within 2 months), and substance use disorder.

The age range of 18–45 was selected to focus on adults capable of providing independent informed consent and reduce developmental variability in neurobiological and psychological measures. While ON may emerge in adolescence, this study targets adult expressions of ON to improve construct validity and control for maturational effects on EEG signals, immune response, and microbiome composition. Future studies may extend the findings to adolescent populations with adapted protocols and additional safeguards.

To strengthen construct validity and reduce reliance on self-report thresholds alone, structured clinical interviews will be expanded to a larger subset of participants (n=80; 40 ON and 40 HO). These will include DSM-5-aligned semi-structured diagnostic interviews, adapted from the EDE-Q and OCI modules, conducted by trained clinical psychologists. We will triangulate psychometric classifications (TOS) with clinical judgments to validate group assignments and detect ambiguous or overlapping cases. Despite prior validation of the Hebrew TOS, we acknowledge that self-report tools may not sufficiently differentiate ON from AN in clinically overlapping cases. To address this, we will triangulate self-report scores with structured clinical interviews and, if needed, refine classification post-hoc using latent profile analysis. We also plan to document ambiguous or ‘gray zone’ cases and analyze them as a distinct subgroup. Importantly, we will conduct Latent Profile Analysis (LPA) across the entire sample—independent of TOS thresholds—to empirically identify ON- and HO-like profiles. These data-driven clusters will be compared to a priori groupings to validate or refine diagnostic classification. This step is central to the study’s goal of mapping construct-relevant patterns, rather than assuming rigid diagnostic boundaries.

Integrating psychometric thresholds and clinical interviews minimizes the risk of circular reasoning and ensures that self-report instrument scores do not solely drive ON/HO group distinctions. Latent profile analyses (LPA) will also be conducted on the entire sample to assess whether ON- and HO-like clusters emerge independently of pre-defined TOS cutoffs.

*Psychological Measures*

Participants will complete a validated battery of psychological instruments:

1. **The Teruel Orthorexia Scale (TOS)** is a 17-item self-report questionnaire that distinguishes between **Healthy Orthorexia** and **Orthorexia Nervosa**. Items are rated on a 4-point scale.
Our lab translated and validated the Hebrew version, confirming its strong reliability and ability to differentiate between healthy and pathological eating behaviours in the Israeli population [6].
2. **The GAD-7** is a brief self-report tool to screen for and assess the severity of generalized anxiety disorder. It measures anxiety symptoms over the past two weeks, with seven items rated on a 4-point scale. Total scores (0–21) indicate severity, with cutoffs at 5 (mild), 10 (moderate), and 15 (severe). The GAD-7 has been translated and validated in Hebrew. It shows excellent reliability (Cronbach's α = .91) and is widely used in Israeli populations for clinical and research purposes [31].
3. **The BDI-II** assesses the severity of depressive symptoms across clinical and non-clinical populations. It is a widely validated self-report instrument consisting of 21 items that reflect the cognitive, affective, and somatic components of depression. This tool will identify progressive symptom profiles and their associations with physiological, behavioral, or psychological variables of interest within the proposed research [32].
4. **The Multidimensional Perfectionism Scale (MPS)** assesses distinct dimensions of perfectionism, including self-oriented, other-oriented, and socially prescribed perfectionism. The MPS provides a nuanced understanding of how perfectionistic tendencies relate to psychopathological outcomes, such as anxiety, depression, and disordered eating, making it a valuable tool for investigating individual differences in vulnerability and resilience [33].
5. **The Obsessive-Compulsive Inventory – Revised (OCI-R)** will be employed to measure the severity and dimensional structure of obsessive-compulsive symptoms across both clinical and subclinical populations. This 18-item self-report scale assesses six symptom domains—washing, checking, ordering, obsessing, hoarding, and neutralizing—offering a reliable and valid tool for identifying obsessive-compulsive tendencies and their associations with related psychological or physiological variables [34].
6. **The Eating Disorder Examination Questionnaire (EDE-Q)** assesses core features of eating disorder psychopathology, including restraint, eating concern, shape concern, and weight concern. As a widely used and psychometrically validated self-report measure, the EDE-Q enables the evaluation of symptom severity and behavioral patterns in clinical and non-clinical populations, supporting the investigation of disordered eating concerning psychological, physiological, and sociocultural variables [35].
7. **The Food Frequency Questionnaire (FFQ)** assesses participants' habitual dietary intake, focusing on food variety and dietary patterns over a defined period. As a standardized and widely used dietary assessment tool, the FFQ enables the identification of nutritional profiles, associations with health outcomes, and dietary influences on psychological and physiological variables relevant to the proposed research [36, 37].

*Immunological Profiling*

To investigate potential immunological mechanisms underlying ON, the study will assess both pro-inflammatory and anti-inflammatory cytokines as markers of immune system balance across four groups: ON, HO, AN, and CG. This approach is grounded in growing evidence linking immune dysregulation to affective, cognitive, and behavioral symptoms observed in eating disorders.

The selected **pro-inflammatory cytokines** include:

* **Interleukin-6 (IL-6)** isan elevated cytokine in malnutrition and chronic psychological stress. It is associated with symptoms such as fatigue, anxiety, and impaired cognitive performance.
* **Interleukin-1β (IL-1β):** A potent mediator of neuroinflammation, often elevated in stress-related conditions and implicated in the disruption of gut-brain communication.
* **Tumor Necrosis Factor-alpha (TNF-α)** is a systemic inflammatory marker associated with mood disorders and anorexia nervosa, involved in appetite regulation and catabolic processes.

To capture regulatory immune responses, two key **anti-inflammatory cytokines** will also be measured:

* **Interleukin-10 (IL-10)** is a critical immunomodulatory cytokine that downregulates inflammation. Lower IL-10 levels may reflect impaired capacity to counterbalance pro-inflammatory responses.
* **Transforming Growth Factor-beta (TGF-β)** is a multifunctional cytokine that maintains mucosal immunity, intestinal barrier integrity, and gut microbiota balance—functions particularly relevant to gut-brain axis dynamics.

Blood samples will be collected in the morning to control for circadian fluctuations in cytokine levels. **All blood draws will be performed by a licensed nurse, specifically trained for the procedure, who will be compensated for their services as part of the study budget.** Samples will be centrifuged to isolate serum, which will be aliquoted and stored at –80°C until analysis. Cytokine concentrations will be quantified using multiplex bead-based immunoassays, such as Luminex xMAP® Technology, allowing simultaneous, high-sensitivity detection of multiple targets in small volumes. This method enhances analytical efficiency and reduces inter-assay variability, participant burden, and sample requirements.

**General Linear Models (GLMs)** will be employed to compare cytokine concentrations across the four groups for statistical analysis. Age, sex, and body mass index (BMI) will be covariates to control for known confounders. If distributional assumptions are violated, cytokine values will be **log-transformed** to improve normality. Additionally, **exploratory cluster analyses** may be conducted to identify biologically meaningful immune subtypes that cut across diagnostic categories, providing insight into transdiagnostic inflammatory profiles potentially relevant to disordered eating phenotypes.

*Neurophysiological (EEG/ERP) Recording*

High-resolution electroencephalography (EEG) will examine the neural mechanisms underlying attentional control, emotional regulation, and self-relevant food processing. ON recordings will be conducted using a **32-channel gTech Nautilus wireless EEG system**, which provides high signal fidelity and participant comfort, essential for capturing naturalistic cognitive and emotional responses.

The EEG assessment will include two components:

1. **Resting-State EEG:** Participants will undergo a 5-minute recording session under both **eyes-open** and **eyes-closed** conditions. These baseline measures allow for the analysis of intrinsic brain activity, including frequency band power (e.g., alpha, theta) and connectivity patterns. Alterations in resting-state dynamics have been previously associated with eating disorders and obsessive-compulsive traits, particularly in individuals with Anorexia Nervosa (AN) and Binge Eating Disorder [12,14,17].
2. **Event-Related Potential (ERP) Task:** Participants will complete the Adult Food Preference Paradigm (AFPP), a cognitive-affective task developed in our lab to probe emotional responses to food-related and self-referential stimuli [38]. Preliminary pilot data will be collected to affirm that the AFPP elicits robust N200, P300, and LPP components concerning health-labelled and emotionally significant food stimuli, alongside completing a validated ERP task used in eating disorder research [e.g., the Food Dot-Probe Task or Food-Categorization Task; , 22]. Full validation and reliability testing will be part of the project’s pilot phase in Year 1. Year 1 will be devoted to full validation of the AFPP, including assessment of reliability and ERP component sensitivity (e.g., N200, P300, LPP). If AFPP fails to meet validation criteria (e.g., low effect size, low internal consistency), a pre-specified fallback task—such as the Food Dot-Probe or Food-Categorization Task, both validated in eating disorder populations—will be used as the primary ERP measure. This ensures data interpretability and comparability to existing literature.

Key ERP components of interest include:

* **N200:** Associated with conflict detection and cognitive control, **particularly relevant for evaluating the neural cost of violating internalized food rules or moral purity ideologies,** as hypothesized in ON.
* **P300:** Reflects attentional allocation and executive processing. **Altered P300 responses in eating disorders have been linked to stimulus salience and self-referential bias, supporting its inclusion as a potential biomarker of ideologically charged dietary restriction**.
* **Late Positive Potential (LPP):** Measures sustained emotional engagement, **which may be heightened in individuals with ON when confronted with norm-violating food images or ambiguous “healthy/unhealthy” categorizations**.

All EEG data will undergo rigorous pre-processing, including **Independent Component Analysis (ICA)** to remove ocular and muscle artifacts, and **bandpass filtering** (0.1–30 Hz). Data epochs will be time-locked to stimulus onset and baseline-corrected for ERP analysis.

Statistical analysis will include **repeated-measures ANOVA** to evaluate group differences in ERP amplitude and latency, with within-subject factors such as stimulus type (e.g., healthy vs. unhealthy food) and between-subject factors (diagnostic group). Additional **time–frequency analyses** (e.g., wavelet transforms) and **source localization techniques** (e.g., sLORETA) may be applied to explore oscillatory dynamics and cortical generators of observed effects.

This neurophysiological component will provide novel insights into how individuals with ON process food and self-relevant stimuli at the neural level. By comparing these responses with those in the HO, AN, and CG groups, the study will help delineate whether ON is marked by unique cognitive-affective processing patterns or shares underlying neural dysfunctions with established eating and obsessive-compulsive disorders.

*Gut Microbiome Analysis*

This microbiome component is exploratory and subject to strict feasibility and interpretability criteria. Stool samples will be collected from a subsample of ~90 participants. Sequencing will proceed only if sufficient DNA quality and funding are available. It will not be included in primary outcome analyses unless Year 2 pilot results demonstrate high DNA yield, robust taxonomic resolution, and adequate group differentiation. If pilot outcomes fall below predefined thresholds (e.g., <80% sample viability, insufficient intergroup variance), this component will be suspended, and funds will be reallocated. Results will be published only as secondary, hypothesis-generating findings, not confirmatory biomarkers.

While microbiome disruptions are a hypothesized correlate of ideologically restrictive eating, this component will be treated as exploratory due to variability in dietary control, lower power for complex community-level comparisons, and resource constraints. Microbiome sequencing findings will primarily inform future mechanistic models and generate hypotheses, rather than test definitive diagnostic criteria. Given its exploratory nature, microbiome-related expenditures (e.g., sequencing, analysis) are contingent and may be scaled back based on pilot findings reviewed in Year 3. A contingency clause in the budget will permit reallocation of these funds toward higher-yield components (e.g., ERP replication or extended follow-up).

To investigate the role of gut microbiota in ON, participants will be asked to collect stool samples using standardized, sterile at-home collection kits provided by the research team. This study component aims to characterize microbiome diversity and composition across individuals with ON, HO, AN, and CG, and to identify microbial signatures potentially associated with restrictive or pathological eating patterns.

Microbial DNA will be extracted from stool samples and subjected to **16S ribosomal RNA (rRNA) gene sequencing**, explicitly targeting the V3–V4 hypervariable regions, which provide robust taxonomic resolution for bacterial community profiling. Sequencing will be performed using Illumina platforms, and raw reads will be processed using the **QIIME2** bioinformatics pipeline, an established open-source platform for microbiome data analysis.

The following analytical approaches will be employed:

* **Alpha diversity metrics** (e.g., Shannon and Simpson indices) will assess within-sample microbial richness and evenness, offering insight into the diversity of bacterial communities within each participant.
* **Beta diversity metrics** (e.g., Bray-Curtis dissimilarity and UniFrac distances) will evaluate between-sample differences in community composition, allowing group-level comparisons across ON, HO, AN, and CG.
* **LEfSe (Linear Discriminant Analysis Effect Size)** will be used to identify differentially abundant taxa between groups, enabling detection of microbial biomarkers with statistical significance and biological relevance.

Beyond taxonomic profiling, exploratory analyses may include predictive functional inference using tools such as PICRUSt2, providing insight into metabolic pathways potentially altered in ON (e.g., those involved in short-chain fatty acid production, inflammation, or neurotransmitter synthesis).

This approach will allow for identifying gut microbial patterns associated with restrictive and ideologically driven eating behaviours. Given the established microbiome disturbances observed in AN and the hypothesized overlap with ON [9–11,21], this analysis may reveal whether ON exhibits similar reductions in diversity and enrichment of pro-inflammatory taxa or reflects a distinct microbial ecology linked to health-focused dietary restriction. These findings will contribute to a broader understanding of how gut-brain interactions may underlie emerging eating disorders in wellness culture.

Additionally, we will not conduct complete metagenomic sequencing due to resource constraints of the microbiome component. Instead, taxonomic profiles will be cautiously interpreted alongside psychometric data. We acknowledge this limitation and treat microbiome results as hypothesis-generating rather than confirmatory. Given the exploratory nature of this component, and the sensitivity of the microbiome to dietary variability, dietary intake will be assessed using the Food Frequency Questionnaire (FFQ) and statistically controlled in microbiome analyses (e.g., via covariate-adjusted PERMANOVA). The inclusion of the microbiome component is justified by ON’s unique dietary rigidity, which may shape microbial ecology in distinct ways. Even as hypothesis-generating, this component offers critical insight into potential gut-brain-immune pathways underlying ideologically driven eating behaviours. In such cases, descriptive analyses and subgroup comparisons will be prioritized, and results will be interpreted cautiously to inform future hypothesis generation rather than confirmatory claims.

*Multimodal Data Integration Strategy*

To ensure feasibility, the multimodal integration phase has been simplified and staged. Initial analyses (PCA, GLMs) will focus on three core domains: psychological measures, EEG data, and cytokine profiles. CCA and clustering will only proceed if adequate variance contribution (>5%) and group differentiation are demonstrated in preliminary analyses. This stepwise approach minimizes overfitting risk and maximizes interpretability.

We will employ three complementary analytical methods to explore cross-domain relationships and identify distinct biopsychological profiles of ON. Principal Component Analysis (PCA) will reduce dimensionality and extract core latent components across domains. Cluster analysis (k-means or hierarchical) will then be applied to identify participant subgroups with shared profiles. Canonical Correlation Analysis (CCA) or Partial Least Squares (PLS) will assess shared variance between biological and psychological markers.

To support this phase, a statistical consultant will be contracted for 12 months across Years 4–5 at 0.25 FTE. Consultant deliverables will include: (1) development of a dimensionality reduction pipeline; (2) stability and validity checks for cluster solutions; and (3) robustness analyses, including cross-validation and outlier detection. A subcontracting plan is outlined in the budget section.

Multimodal integration (PCA, clustering, CCA) will be conducted primarily across psychological, immunological, and EEG domains. Microbiome data will be incorporated only if sequencing quality, sample size, and statistical variance contribution meet predefined thresholds (e.g., >5% variance explained in PCA components).

We recognize that the integration of four high-dimensional datasets presents substantial complexity. To mitigate this, integration will proceed in stages, starting with psychological, EEG, and cytokine data. Microbiome data will be included only if sequencing quality, taxonomic resolution, and contribution to explained variance meet predefined thresholds. This staged approach ensures interpretability and minimizes the risk of spurious or uninterpretable findings due to noisy or low-yield components.

All analytic thresholds, component inclusion rules, and planned analyses will be pre-registered on the Open Science Framework (OSF) at project onset. Criteria for proceeding with full multimodal integration include: (a) ≥10% explained variance for principal components, (b) ≥.70 reliability for EEG/ERP components, and (c) minimum 5% unique contribution from each domain in preliminary PCA. If any domain fails to meet these thresholds, it will be excluded from CCA or clustering analyses. This pre-registration plan prioritizes primary hypotheses and safeguards against exploratory overreach.

*Enhanced Integration Plan and Expert Support*

Recognizing the complexity of integrating psychological, immunological, microbiome, and neurophysiological data, we have expanded our integration strategy into a structured, phased plan.

* **Years 2–3:** Data harmonization and dimensionality reduction (e.g., factor analysis, ICA, taxonomic aggregation) across domains.
* **Years 3–4:** Multivariate and clustering techniques (e.g., CCA, PLS, latent profile analysis) will model cross-domain phenotypes.
* **Year 5:** Predictive validity testing and robustness checks using longitudinal data.

*Expert Consultation*

To enhance statistical and methodological integrity, we will formally engage a biostatistics consultant specializing in multimodal integration and machine learning in clinical research. This individual will participate in model development, validation, and interpretation stages, ensuring data coherence.

The theoretical contribution of this integration plan lies in its capacity to uncover latent profiles of Orthorexic behavior that are not detectable through single-domain analyses. For example, clustering may reveal distinct ON subtypes — such as 'inflammatory-rigid' vs. 'anxious-restrictive' — that differ in biological and psychological configuration. PCA and CCA will enable us to explore whether psychological traits like perfectionism and obsessive-compulsive tendencies align with specific neurophysiological (ERP) and immunological markers, providing empirical support for transdiagnostic models of ON. Ultimately, these multivariate patterns will inform whether ON is best understood as a unitary syndrome, a spectrum of ideologically-driven behaviours, or a biopsychological phenotype overlapping with AN and OCD. This insight is essential for refining dimensional models of ON and guiding the development of tailored prevention and intervention strategies.

Should data complexity exceed projections, we are prepared to increase consultant support using flexible contingency funds already built into the budget. Additionally, one PhD student will be trained in R and multivariate modelling under the consultant's guidance to support longer-term data integration and reduce reliance on external personnel.

*Longitudinal Follow-Up*

A subset comprising approximately **30% of participants from each group** (ON, HO, AN, and CG) will be **reassessed to evaluate the temporal stability and predictive utility of key psychological and biological markers after 12 months**. This follow-up phase will investigate whether observed profiles—particularly those related to inflammation, microbiome composition, neural activity, and psychological traits—represent stable, trait-like characteristics or fluctuate over time concerning symptom severity or recovery trajectories.

Participants will undergo the same battery of assessments as at baseline, including psychological questionnaires, blood sampling for cytokines, stool collection for microbiome analysis, and EEG recording. This repeated-measures design is essential for capturing intra-individual variability and identifying biomarkers with potential prognostic value.

To reduce attrition, we will implement: (a) biannual check-ins via SMS/email, (b) flexible scheduling, (c) transportation stipends, and (d) bonus compensation upon study completion. Prior lab studies using similar protocols achieved >85% retention at 6–12 months.

**Linear mixed-effects models (LMMs)** will be used to analyze longitudinal data, offering a robust statistical framework for handling repeated measures, missing data, and individual variability. These models will assess both **within-subject changes** over time and **between-group differences** in the evolution of biological and psychological variables. Importantly, LMMs will also allow identifying baseline features, such as elevated IL-6 or altered ERP patterns, that predict persistence, remission, or escalation of orthorexic symptoms over time.

This longitudinal component enhances the scientific and clinical significance of the project by moving beyond static cross-sectional comparisons to a more dynamic understanding of ON’s trajectory and its biological correlates. Findings may inform the development of **early identification tools**, **risk models**, and **targeted intervention strategies** for individuals vulnerable to developing or maintaining pathological health-related eating behaviours.

**Data Analysis Plan**

The proposed study will generate a rich, multimodal dataset spanning psychological, immunological, microbiome, and neurophysiological domains. A rigorous and tailored statistical approach will be applied to each domain to maximize the data's interpretability and scientific value. Integrative analyses will follow this to identify transdiagnostic patterns and potential ON biomarker signatures.

1. **Psychological Data:** Multivariate analyses of variance (**MANOVA**) will be used to assess group differences across psychological constructs, including anxiety, perfectionism, obsessive-compulsive traits, and disordered eating symptoms. Where significant multivariate effects are found, **Bonferroni-adjusted post-hoc comparisons** will identify specific group contrasts (e.g., ON vs. HO, ON vs. AN).
2. **Immunological (Cytokine) Data:** **General linear models (GLMs)** will be employed to evaluate between-group differences in pro- and anti-inflammatory cytokines (e.g., IL-6, TNF-α, IL-10) while controlling for relevant covariates such as age, sex, and BMI. Cytokine values will be **log-transformed** to correct for non-normal distributions and heteroscedasticity. Menstrual cycle phase, sleep quality, and medication use will be recorded and included as covariates in all cytokine and EEG analyses. All blood draws will occur between 8-10 am after fasting.
3. **Gut Microbiome Data:** Community-level differences in microbiome composition will be analyzed using **Permutational Multivariate Analysis of Variance (PERMANOVA)** on beta diversity metrics (e.g., Bray-Curtis, UniFrac). We will use **LEfSe (Linear Discriminant Analysis Effect Size) to identify taxonomic features that differentiate diagnostic groups**, which combines statistical significance with biological relevance to highlight discriminatory taxa.
4. **Neurophysiological (EEG/ERP) Data:** EEG data will be analyzed using **repeated-measures ANOVA** to examine group differences in ERP components (N200, P300, LPP) across stimulus types. Additional analyses will include **time–frequency decomposition** (e.g., wavelet transforms) to explore changes in oscillatory power, and **topographical mapping** to examine spatial distributions of neural activity. Advanced analyses such as **source localization** (e.g., sLORETA) may be applied to identify cortical generators underlying ERP effects. EEG sessions will be conducted at fixed times (10 am-2 pm) to control for diurnal variation. Rest periods and noise calibration will minimize fatigue and artifacts.
5. **Longitudinal Data:** For the 12-month follow-up subsample, linear mixed-effects models (LMMs) will be used to model within-person change over time and examine the predictive value of baseline markers (e.g., cytokines, ERP features, microbiome diversity) for clinical outcomes. LMMs offer robust handling of repeated measures and missing data, making them ideal for longitudinal modelling.

This comprehensive and multi-tiered analytical strategy ensures that the study can detect both group-level differences and nuanced transdiagnostic patterns, ultimately contributing to a refined, biologically grounded model of ON. Analytical thresholds for model inclusion will be pre-registered (e.g., ≥10% variance explained for PCA components; reliability coefficients ≥.70 for ERP measures). Multivariate corrections (e.g., FDR control, Bonferroni adjustment) will be applied to reduce false discovery risk in all hypothesis-testing analyses.

**Preliminary Results**

Figure 1 presents our preliminary findings indicating a consistent and meaningful pattern: higher ON symptoms levels are associated with lower scores across several key dimensions of psychological and embodied well-being. Specifically, individuals with more pronounced orthorexic tendencies report reduced body appreciation, lower levels of intuitive eating, decreased embodiment, and a diminished sense of control. These results suggest that, contrary to popular assumptions, orthorexic behaviours—often perceived as disciplined or health-promoting—may compromise one’s psychological flexibility, body trust, and regulatory capacities [6].

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| **Figure 1**. Pearson correlation coefficients between Orthorexia Nervosa and four psychological constructs: Experience of Embodiment (r = –.49), Sense of Control (r = –.29), Body Appreciation (r = –.46), and Intuitive Eating (r = –.54). All correlations were statistically significant at p < .01, indicating that higher ON levels are associated with lower levels of these positive psychological constructs. |

While we have not yet conducted pilot biological sampling due to resource and timing constraints, the proposed methodology is grounded in robust, peer-reviewed literature. Prior studies have demonstrated elevated pro-inflammatory cytokines (IL-6, TNF-α) in AN and OCD, both of which share key traits with ON, such as perfectionism, cognitive rigidity, and anxiety [10, 12]. EEG studies in AN population have also revealed reduced P300 amplitudes and altered N200 responses during food-related decision-making tasks, reflecting attentional and emotional dysregulation [22, 23]. These findings support the feasibility of our proposed biomarkers and reinforce the likelihood that ON may exhibit comparable, yet distinct, biological signatures.

**Facilities and Resources Available for the Execution of the Research**

The proposed research will be conducted at the **Laboratory of Physiology and Behaviour—Nutritional Psychology** at **Tel-Hai Academic College**, under the direction of **Dr. Omer Horovitz** (Principal Investigator). The laboratory is fully equipped to execute the proposed multimodal study, which integrates psychological, neurophysiological, immunological, and microbiome methodologies.

The lab infrastructure includes:

* Two **32-channel g.Tec Nautilus wireless EEG systems are** suitable for high-resolution resting-state and ERP recordings.
* A **sound-attenuated experimental room**, optimized for EEG recordings under controlled conditions.
* Access to advanced **computational infrastructure**, including data servers and software for signal processing, statistical modelling (e.g., MATLAB, SPSS, R, QIIME2).
* Established collaborations with laboratories (i.e., Prof. Snait Tamir’s Lab at MIGAL) will ensure high-quality, domain-specific supervision and processing of cytokine and microbiome samples.

The project team will also include three graduate research students (two M.A. and two Ph.D. candidates) who will assist with data collection, pre-processing, and analysis across the project's domains. EEG and ERP acquisition, pre-processing (e.g., ICA, filtering, epoching), and interpretation will be led and supervised directly by the PI, who has extensive expertise in EEG methods, including a published ERP study on cognitive-affective processing [39]. This ensures that the neurophysiological component of the project will be conducted with scientific rigor and without the need for external supervision or consultants.

Dr. Horovitz has extensive experience supervising graduate students and leading interdisciplinary research programs, which ensures that the proposed project will be executed with scientific rigor, administrative efficiency, and high-quality mentorship.

**Expected Results and Potential Pitfalls**

*Expected Results*

The proposed study is expected to yield novel, integrative findings that will significantly advance our understanding of ON as a potential distinct clinical entity. Specifically, we anticipate the following outcomes:

1. **Psychological Profile**: Individuals with ON will demonstrate significantly higher levels of perfectionism, anxiety, obsessive-compulsive traits, and cognitive rigidity compared to HO and healthy controls, and in patterns partially overlapping with AN.
2. **Immunological Findings**: ON participants will show elevated pro-inflammatory cytokines (e.g., IL-6, IL-1β, TNF-α) relative to HO and CG, though likely to a lesser extent than in AN, suggesting a gradient of immune activation across groups.
3. **Microbiome Signatures**: ON will be associated with reduced microbial diversity and specific pro-inflammatory taxa, reflecting restrictive dietary patterns. Distinct microbial profiles may emerge that differentiate ON from AN and HO.
4. **Neurophysiological Markers**: ERP components (N200, P300, LPP) will reflect altered attentional and emotional processing in ON, particularly in response to food and self-related stimuli, with patterns resembling but not identical to those observed in AN.
5. **Longitudinal Predictors**: Follow-up data will inform whether baseline biological and psychological markers predict persistence or remission of orthorexic behaviours, supporting potential prognostic tools.

*Potential Pitfalls and Alternative Strategies*

While the project is grounded in robust preliminary data and relies on validated methods, several challenges may arise:

1. **Recruitment of Clinical Participants (AN group)**

*Pitfall*: Difficulty in recruiting sufficient numbers of participants with a formal AN diagnosis.
*Mitigation*: Collaborations with local eating disorder clinics and university counselling centers are already in place. Oversampling in the HO and ON groups will allow for adjusted comparisons if AN numbers are lower.

1. **EEG Data Quality**

*Pitfall*: EEG data may be affected by motion artifacts or participant fatigue.
*Mitigation*: Extensive technician training, standardized protocols, and pre-screening for suitability will ensure data integrity. ICA and artifact correction methods will also help.

1. **Variability in Microbiome Samples**

*Pitfall*: Dietary or environmental factors may introduce noise in microbiome data.
*Mitigation*: Clear instructions for standardized stool sample collection, dietary recall control (FFQ), and exclusion of recent antibiotic users.

1. **Cytokine Assay Sensitivity**

*Pitfall*: Low cytokine concentrations may challenge detection.
*Mitigation*: Using highly sensitive multiplex bead-based assays (e.g., Luminex xMAP) and batch processing to minimize variability.

1. **Interpretation of Multimodal Data**

*Pitfall*: High dimensionality and complexity may limit clear interpretation.
*Mitigation*: Expert consultation in biostatistics, dimensionality reduction, and unsupervised clustering will support robust, interpretable findings.

1. **Participant Attrition in Follow-Up**

*Pitfall*: Loss of participants at the 12-month follow-up may reduce statistical power.
*Mitigation*: Over-recruitment and use of participant engagement strategies (e.g., reminders, flexible scheduling, incentives) to maximize retention.

1. **Analytic Overreach and Scope Creep**

*Pitfall:* Integrating four high-dimensional data modalities in a single project risks overextension and diluted interpretability.

*Mitigation:* The project has been refocused around four core domains (psychology, immunology, EEG, microbiome). The data will only be integrated into a model when data quality and statistical power allow.

All data modelling decisions—including exclusion thresholds, feature selection, and analytic criteria—will be transparently documented in a public pre-registration. Secondary outcomes will be excluded from primary publication or integration models if effect sizes fall below Cohen’s d = 0.3 or statistical power is < 0.70. This ensures analytical transparency and guards against confirmatory bias.

Given the complexity and resource demands, microbiome results will be treated as hypothesis-generating and may be omitted from primary outcome publications pending data quality and power. By proactively addressing these potential obstacles and integrating flexible, validated data collection and analysis strategies, the project is well-positioned to achieve its objectives and make a meaningful contribution to psychiatric and nutritional science.

**Work plan**

The project is structured over five years and is divided into clearly defined and interdependent phases. Each phase aligns with the study's objectives and methodological components, including participant recruitment, multimodal data collection (psychological, immunological, microbiome, EEG/ERP), longitudinal follow-up, and comprehensive data analysis. The timeline below outlines the major tasks and milestones for each project year. Tasks will be carried out by a dedicated interdisciplinary team, including the PI, graduate students, and trained research technicians. Parallel data collection and analysis streams will be implemented to maximize efficiency and ensure timely progress (please see Figure 2).

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| **Figure 2.** Work Plan Timeline - The Gantt chart displays the scheduled timeline for each project phase across a 60-month period. Bars represent the duration (in months) of key research activities, including protocol finalization, data collection, follow-up assessments, data analysis, and dissemination. The chart facilitates visual tracking of task overlap, sequence, and duration, supporting efficient project planning and execution |

**Research Timeline and Phases**

The proposed project is structured across five years, each year dedicated to clearly defined and interdependent stages. The timeline reflects the methodological complexity of the study, including participant recruitment, multimodal data collection (psychological, immunological, microbiome, and EEG/ERP), longitudinal follow-up, data pre-processing, statistical analysis, and dissemination of results.

A core team, including the PI, graduate students, trained technicians, and clinical collaborators, will ensure the timely execution of each phase. Parallel processing of biological and EEG data will be implemented to optimize efficiency. The following table presents specific milestones and deliverables at each project stage, offering transparency into when key datasets, analyses, and outputs will be completed (see Table 1).

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| --- | --- | --- | --- |
| **Year** | **Months** | **Phase** | **Activities** |
| Year 1 | 1–6 | Preparation | Finalize study protocol, ethics approval obtained, EEG system tested and calibrated |
|  | 7–12 | Extended Pilot | Pilot complete for 20 participants (EEG, cytokine, microbiome, psychometrics); finalize ERP paradigm |
| Year 2 | 1–12 | Recruitment Phase I | Recruit and assess 50% of total sample (n=150); begin cytokine and EEG data acquisition |
| Year 3 | 1–12 | Recruitment Phase II | Complete recruitment (n=300); complete psychological and EEG/ERP testing for all participants |
| Year 4 | 1–6 | Completion of Data Collection | By Month 42, cytokine assays will be completed. Microbiome sequencing will only be initiated if pilot data (e.g., sample integrity, DNA yield) and funding availability support meaningful exploratory analysis |
|  | 7–12 | Longitudinal Follow-Up | Conduct 12-month follow-up for ~30% of sample (n≈90); initiate EEG and biological retesting |
| Year 5 | 1–8 | Data Processing & Analysis | EEG/ERP pre-processing complete (Month 52); cytokine/microbiome integration complete (Month 54) |
|  | 9–12 | Dissemination | Final publications submitted; policy briefs prepared; Ministry of Health dissemination initiated |

**Budget**

The requested budget will ensure the successful and timely completion of all study phases. Costs are distributed across equipment maintenance, biological assays, participant engagement, student support, and knowledge dissemination activities. Most funding supports direct research costs, ensuring maximal scientific output relative to investment (please see Table 2).

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| --- | --- | --- | --- | --- | --- | --- | --- |
| **Category** | **Year 1 (NIS)** | **Year 2 (NIS)** | **Year 3 (NIS)** | **Year 4 (NIS)** | **Year 5 (NIS)** | **Total (NIS)** | **Details** |
| EEG System (2 existing) | ₪0 | ₪0 | ₪0 | ₪0 | ₪0 | ₪0 | Already available |
| EEG Consumables | ₪3,700 | ₪3,700 | ₪3,700 | ₪3,700 | ₪0 | ₪14,800 | Electrodes, gels, caps, cables |
| Computers/Software | ₪18,500 | ₪0 | ₪0 | ₪5,000 | ₪5,000 | ₪28,500 | Analysis software updates, computational needs |
| Microbiome Collection Kits\* | ₪0 | ₪0 | ₪7,400 | ₪3,700 | ₪7,400 | ₪18,500 | DNA extraction, storage, sequencing |
| Blood Biomarker Kits | ₪18,500 | ₪18,500 | ₪18,500 | ₪7,400 | ₪0 | ₪62,900 | Cytokine assays, immune profiling kits |
| Participant Incentives | ₪27,750 | ₪27,750 | ₪18,500 | ₪9,250 | ₪0 | ₪83,250 | ~₪185 per participant across ~450 participant-visits |
| Travel for Presentations | ₪0 | ₪9,250 | ₪9,250 | ₪9,250 | ₪9,250 | ₪37,000 | National and international conference participation |
| Contingency Fund | ₪6,167 | ₪6,167 | ₪6,166 | ₪6,166 | ₪6,166 | ₪30,832 | Unforeseen costs (sample loss, repairs, replacements) |
| Statistical/Biostatistical Consultant | ₪0 | ₪0 | ₪0 | ₪9,250 | ₪9,250 | ₪18,500 | Support for multimodal integration (CCA, PCA, clustering) in Year 4–5 |
| 2 MA Students (part-time) | ₪0 | ₪60,000 | ₪60,000 | ₪30,000 | ₪0 | ₪150,000 | Assistance in data collection and analysis |
| 2 PhD Students (full-time) | ₪72,000 | ₪72,000 | ₪72,000 | ₪72,000 | ₪72,000 | ₪360,000 | Full stipend (~₪6,000/month/student) |
| **Total Estimated Costs** | **₪145,617** | **₪197,367** | **₪195,516** | **₪155,716** | **₪109,066** | **₪803,282** |  |

\* To improve budget efficiency and reduce risk, microbiome-related expenditures (e.g., sequencing, extraction kits) are designated conditional Year 3 expenditures, pending results from the pilot microbiome substudy. Should findings not support adequate statistical power, sample viability, or group-level differentiation, these funds will be reallocated to support higher-impact components such as ERP replication, advanced data integration, or student training. This tiered funding approach ensures responsible use of exploratory research funds.

We acknowledge the concentration of biological expenses in Year 3 due to overlapping cytokine and microbiome activities. To reduce risk of cost overruns due to sample loss or attrition, buffer funds are included in the contingency line. Furthermore, microbiome analyses will proceed only after pilot quality thresholds are met, and sequencing expenses can be reallocated if thresholds are not achieved.

In sum, this project offers a unique opportunity to advance scientific understanding, clinical diagnostics, and public health interventions related to Orthorexia Nervosa, positioning Israel at the forefront of global research on emerging mental health challenges.

**Principal Investigator Time Commitment and Salary Declaration**

The Principal Investigator, **Dr. Omer Horovitz**, will dedicate **30% of his time** to the execution, supervision, and scientific management of this research project throughout its five-year duration.

**No salary support is requested** for the Principal Investigator or any other senior academic staff member who holds an academic appointment at their institution. By the funding agency's guidelines, no portion of the requested budget will be used to cover salaries for researchers eligible to submit applications as Principal Investigators in any of the Foundation’s funding tracks.

As outlined in the detailed budget justification, all budgetary allocations are designated solely for direct research expenses, including research assistants, graduate student stipends, equipment, consumables, and dissemination costs.

**Equipment and Supplies**

The following items are requested under this category, each justified according to the research needs:

1. **Computer and Related Equipment (Year 1 & Year 4)**: A high-performance computer workstation and software licenses (e.g., MATLAB, QIIME2-compatible tools) are requested to support processing large EEG/ERP datasets and microbiome sequencing data. While basic computing infrastructure exists at the lab, the volume and complexity of multimodal data (including time-series neurophysiological signals and next-generation sequencing outputs) require enhanced computational capacity to ensure efficient analysis and secure data storage.
2. **Participant Compensation (Years 1–4)**: Financial compensation is requested for participants who complete multiple stages of the study, including psychological assessments, EEG recordings, blood draws for cytokine analysis, and stool sample collection. Compensation (averaging ~185 NIS per participant per session) is essential for ensuring compliance, enhancing retention, particularly for the longitudinal follow-up, and acknowledging participant time and effort.
3. **Graduate Student Travel Allowance (Years 2–5)**: An annual allocation of up to 6,000 NIS is requested to support conference travel or scientific workshops for the two graduate students (M.A. and Ph.D.) employed on this project. This funding will enable them to present findings at national and international meetings and gain methodological training in psychophysiology, immunology, or microbiome analysis. By the foundation’s guidelines, this allocation will be used **only by students**, not by the Principal Investigator.
4. **Miscellaneous (up to 10% of total annual budget)**: A modest allowance is requested for unanticipated, essential research-related expenses such as equipment maintenance, replacement of consumables (e.g., EEG electrodes or reagent kits), or professional translation services for publications. This allocation will be managed prudently and transparently, with complete documentation provided in financial reports.

All requested items directly support the goals and execution of the proposed multimodal investigation of Orthorexia Nervosa. Without prior approval, no items beyond those described here will be purchased using this grant.

**Miscellaneous Expenses**

This budget category includes essential research-related services and operational costs that directly support the successful execution and dissemination of the proposed study. All expenses listed below are relevant to the research plan and comply with the foundation’s regulations. The total amount requested under this section will **not exceed 15% of the annual budget** in any given year.

The following items are included:

1. **Professional Literature**: A limited allocation is requested to purchase up-to-date professional books and reference materials not currently available in the Tel-Hai Academic College library or the National Library of Israel. These resources support the project's interdisciplinary focus in nutritional psychology, psychoneuroimmunology, and eating disorders.
2. **Publication Fees and Language Editing**: Funds are requested to cover article processing charges (APCs) for publishing findings in open-access scientific journals and professional English **editing and translation services**, when required, to meet journal standards. These services will ensure the clarity, accessibility, and international dissemination of the research outcomes.
3. **Printing, Photocopying, and Office Supplies**: Modest expenses are included for routine printing, photocopying, and purchasing consumable office materials (e.g., files, paper, data storage devices) necessary for participant management and documentation.
4. **Internet and Software Access**: Costs associated with high-speed internet connectivity and access to cloud-based research platforms may be included, where relevant to data sharing and remote collaboration.
5. **Membership in Scientific Societies**: Funding is also requested for annual membership fees in relevant **professional associations** (e.g., International Society for Nutritional Psychiatry, Society for Psychophysiological Research), which would enable the research team to stay current with current methodologies, attend member-only conferences, and access research tools and publications.

All expenses in this category are modest, fully justified, and essential for the research project’s operation, dissemination, and scholarly integration. No unrelated administrative costs or institutional overheads will be charged to this grant.

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