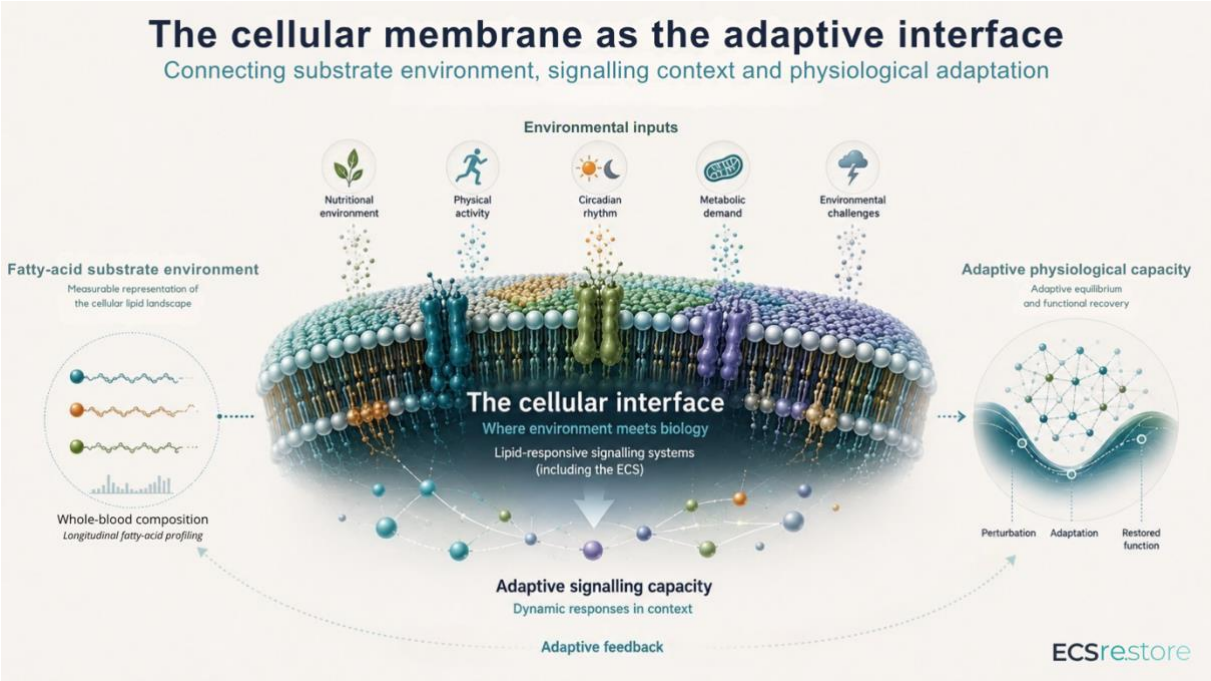
Longitudinal Fatty-Acid Remodeling as a Measurable Biological State Variable

A substrate-driven framework for understanding lipid composition, cellular signalling environment, and physiological adaptation

SCIENTIFIC WHITE PAPER



Graphical Abstract. The cellular membrane as the adaptive interface between environment and physiology. This conceptual framework proposes that biological adaptation emerges from the interaction between environmental inputs, cellular substrate availability, membrane organization, and context-dependent signalling processes. The cellular membrane is presented as a dynamic interface where the biochemical environment influences the context in which lipid-responsive signalling systems operate, including the endocannabinoid system (ECS). Longitudinal whole-blood fatty-acid profiling provides a measurable representation of changes in the lipid substrate environment, while downstream adaptive signalling capacity and physiological adaptation represent areas for future investigation. The framework does not imply that fatty-acid composition alone determines physiological outcomes. Rather, it proposes that lipid composition represents one measurable component of the cellular environment that may contribute to the regulation of adaptive biological responses.

1. Introduction

Biology is often measured by what a system produces. Less often measured is the environment that determines how biological information is received, organized, and translated into response.

The missing dimension in biological measurement

Modern biology has become exceptionally powerful at describing its component parts. Genomics maps inherited potential. Proteomics and metabolomics reveal active molecular processes. Imaging shows where structures are located, and clinical biomarkers help identify when physiology has moved outside an expected range. Together, these tools have transformed our ability to characterize disease, follow molecular pathways, and compare biological states.

Yet a central layer remains comparatively difficult to see: the cellular environment in which signals are interpreted. Cells do not respond to information in isolation. Receptors, enzymes, transporters, and signalling networks operate within a biochemical setting that is continually shaped by substrate availability, membrane lipid composition, metabolic demand, inflammatory tone, receptor organization, and intracellular architecture. The presence of a signalling molecule is therefore only part of the story. The same signal may be interpreted differently when the surrounding biological context changes.

This matters because adaptation is not a property of one molecule. It emerges from relationships across multiple layers of organization. A systems biology view therefore asks not only which components are present, but how the state of the system conditions what those components can do. The relevant unit of inquiry begins to shift from an isolated marker toward the configuration in which many markers coexist. [1]

Beyond receptor-centric biology

A large part of modern pharmacology has been built around a receptor-centric sequence: a ligand binds a receptor, the receptor activates a pathway, and the pathway produces a response. This framework has generated enormous scientific and therapeutic progress. It is clear, testable, and often highly productive. But it can also encourage us to treat the receptor as though it were operating on a neutral stage.

The stage is not neutral. Receptors are embedded within membranes, positioned within local lipid domains, coupled to interacting proteins, and exposed to a changing supply of substrates and signalling precursors. Their behaviour depends not only on molecular identity but also on localization, organization, timing, and metabolic context. In this perspective, the membrane is not simply where receptors reside. It is part of the information-processing system that helps determine how receptors function. [2–7]

> **The biological question is not only whether a signalling pathway exists, but whether the cellular environment allows that pathway to function within an adaptive context.**

The membrane as the adaptive interface

The cellular membrane occupies a distinctive position between environment and physiology. It separates compartments, but it also connects them. Nutritional exposure, physical activity, circadian rhythm, metabolic demand, inflammatory processes, and other environmental pressures can all influence the biochemical conditions from which membrane organization emerges. At the same time, receptors and signalling machinery positioned within or near the membrane translate those conditions into cellular responses.

This makes the membrane more than a structural boundary. It is a dynamic interface in which lipid composition, receptor context, molecular traffic, and signalling capacity converge. Its organization is neither fixed nor infinitely flexible. It is continuously remodeled, yet constrained by prior state, substrate availability, enzymatic control, and physiological demand. The membrane therefore offers a biological meeting point between what the organism is exposed to and how the organism is able to respond. [2–5]

This White Paper is anchored in that interface. The central proposal is not that membrane composition alone determines physiology. It is that the membrane represents a missing explanatory layer: a context in which environmental inputs can alter the conditions under which signalling systems operate. Measuring one aspect of that context may provide a new way to observe biological adaptation without reducing adaptation to a single pathway or outcome.

The endocannabinoid system as adaptive lipid signalling

The endocannabinoid system (ECS) provides a particularly useful example. It is often introduced through cannabinoid receptors or the pharmacology of cannabis-derived compounds. Yet biologically, the ECS is an endogenous lipid signalling network. Its activity depends on the availability of lipid-derived precursors, the enzymes that synthesize and degrade signalling molecules, the localization and organization of receptors, and the downstream networks through which signals are interpreted. [8–10]

The ECS is therefore not adequately described as a receptor-ligand system alone. It can be understood as a demand-responsive regulatory network whose signalling capacity is shaped by the cellular environment in which it operates. This does not mean that a fatty-acid profile measures ECS function, nor that a change in lipid composition demonstrates ECS activation. It means that ECS biology makes the broader principle unusually visible: signalling emerges from an interaction between molecular machinery and the substrate-rich membrane context that supports it.

The question consequently changes. Instead of asking only, ‘Which molecule activates this receptor?’, a substrate-driven perspective also asks, ‘What biological environment determines how that receptor interprets signals?’ This second question does not replace receptor pharmacology. It extends it into the systems context in which endogenous signalling actually occurs.

From fatty-acid biomarkers to biological state variables

Fatty acids offer a practical entry point into this otherwise difficult-to-measure layer. They are membrane constituents, metabolic substrates, precursors for bioactive lipids, and participants in broader regulatory networks. Their relative abundance reflects the combined influence of exposure, absorption, transport, incorporation, utilization, and remodeling. A whole-blood fatty-acid profile is therefore not a direct image of a cellular membrane, but it can provide a measurable representation of the lipid substrate environment from which membrane organization and lipid-responsive signalling emerge. [2–5,11,12]

Traditional interpretation often focuses on individual markers: the omega-3 index, the abundance of a particular fatty acid, or a ratio between selected lipid classes. These measurements can be valuable. But a systems perspective asks a different question: does the overall fatty-acid composition occupy a measurable state that changes over time? A biomarker reports a value. A state variable helps describe the condition of a dynamic system. The distinction is important because coordinated change across multiple fatty acids may carry information that is not visible when each component is considered separately.

In this framework, the fatty-acid landscape is treated as a multidimensional configuration rather than a collection of isolated numbers. Movement through that landscape does not automatically indicate improvement or deterioration. It indicates that the substrate environment has become compositionally different. Biological meaning must then be established through independent functional measurements, controlled interventions, and replication in larger cohorts.

From static measurements to dynamic biological states

Most clinical measurements are interpreted as snapshots. They answer a necessary question: what is the status today? Adaptation, however, is revealed by movement. It involves transitions between metabolic conditions, membrane configurations, signalling contexts, and physiological demands. A longitudinal series can therefore show something that a single measurement cannot: direction, magnitude, timing, stability, and the possibility of an emerging equilibrium.

The present exploratory longitudinal case study uses serial whole-blood fatty-acid profiling across four time points—June 2025, November 2025, February 2026, and June 2026—to explore whether the lipid composition remained static or moved through distinguishable configurations. The analysis does not ask whether one fatty acid rose or fell in isolation. It examines the relationships among measured fatty acids and the coordinated pattern of change across the observation period.

The figures develop this argument progressively. Longitudinal trajectories first show what changed. Principal component analysis then places each time point within multidimensional fatty-acid composition space. Hierarchical clustering provides an independent view of similarity between configurations. Transition vectors examine the direction and relative contribution of individual fatty acids, and lipid-class decomposition reveals how the final transition was distributed across broader compositional classes. Together, these views treat time not as background information, but as a primary biological dimension.

A hypothesis-generating systems biology perspective

This White Paper presents an exploratory framework rather than a mechanistic conclusion. The dataset is observational, small, and centered on one longitudinal individual. Multiple nutritional, training, and physiological factors changed over the same period. Whole-blood fatty-acid composition does not directly measure membrane organization, endocannabinoid tone, receptor behaviour, or physiological resilience. Those limitations define what can—and cannot—be inferred from the figures that follow.

Within those boundaries, the analysis tests a focused proposition: longitudinal fatty-acid composition may function as a measurable biological state variable that reflects change in the cellular substrate environment. If that proposition proves useful, fatty-acid profiling could become one layer in a broader adaptive-state framework, integrated with autonomic, metabolic, sleep, inflammatory, and functional signalling measurements.

The larger scientific opportunity is not another static biomarker. It is a dynamic view of biological context: a way to study how environmental inputs, lipid substrate state, membrane organization, signalling capacity, and physiology may relate across time. The present observations are intended to make that framework visible and to motivate the controlled studies required to test it.

2. Beyond receptor-centric biology: the substrate-driven ECS framework

The receptor is indispensable, but it is not the whole system. A receptor receives a signal from within a membrane, inside a cell, under conditions shaped by substrate availability, metabolic demand, and prior biological state.

The power—and the boundary—of the receptor-centric lens

Traditional pharmacology often begins with a precise and productive question: what ligand activates this receptor? The question has organized decades of discovery. It allows molecular targets to be identified, binding affinities to be measured, dose-response relationships to be described, and therapeutic compounds to be designed with increasing specificity.

The strength of this approach is reduction. It isolates a relationship so that it can be tested. Its limitation is also reduction. Once the receptor is removed from its living context, the experimental clarity can make the surrounding biology appear secondary—even when that surrounding biology is part of what determines the response.

In the organism, a receptor is never simply a free-standing switch. It is embedded within a membrane, positioned within a local lipid environment, connected to accessory proteins, and coupled to intracellular machinery. Its availability, mobility, clustering, conformation, internalization, and downstream coupling can all vary with cellular state. Ligand and receptor remain essential, but neither acts on a neutral stage. [6,7]

> **Traditional pharmacology asks: What ligand activates this receptor?**
>
> **The substrate-driven perspective asks: What biological environment determines how that receptor interprets signals?**

These are not competing questions. The first identifies molecular interaction. The second places that interaction back inside the system. Together, they move the analysis from receptor activation toward signalling competence: not only whether a pathway can be engaged, but how the surrounding cellular conditions shape the form, timing, and consequence of that engagement.

Why the ECS makes context impossible to ignore

The endocannabinoid system (ECS) is especially suited to this wider view because its biology resists clean separation from the lipid environment. The ECS is not simply a cannabinoid receptor system. It is an endogenous lipid signalling network whose function depends on lipid precursors, membrane organization, receptor localization and downstream signalling context. [8–10]

That sentence is the conceptual hinge. It shifts attention away from an ECS defined primarily by CB1, CB2, or externally administered cannabinoids and toward an endogenous regulatory system that is assembled and deployed within living tissue. The receptors matter. So do the enzymes, transport processes, lipid precursors, membrane domains, cellular compartments, and physiological demands that determine when and where signalling becomes possible.

This is why the ECS should not be treated here as a catalogue of receptors, ligands, and enzymes. The purpose is not to reproduce an ECS review. The purpose is to use the ECS as a clear biological

example of a broader principle: signalling capacity is shaped by the environment from which the signal emerges and the membrane context in which it is received.

Lipid-derived

Endocannabinoid signalling begins with lipids. Its principal endogenous mediators are synthesized from lipid precursors and act within a landscape structured by lipid availability and metabolism. This does not mean that the abundance of a precursor maps directly onto signalling output. Synthesis and degradation are enzymatically controlled; mediators are produced and cleared in specific compartments; and the same substrate may participate in multiple competing pathways. [8,10]

The important point is more fundamental. In a lipid-derived signalling system, the substrate environment cannot be regarded as an inert background. It helps define the material conditions from which signalling molecules can be generated. The composition of that environment is therefore relevant to biological capacity even when it is not sufficient to predict a particular response.

Demand-driven

The ECS is commonly described as operating on demand. This is a useful phrase, but it can become vague unless demand is situated biologically. Demand arises when cells and tissues encounter changing conditions: altered energy balance, stress, inflammation, neural activity, injury, feeding, sleep-wake transitions, or other perturbations that require regulation rather than a fixed output.

An on-demand system must therefore do more than contain the necessary molecules. It must be able to mobilize them with appropriate timing, location, and duration. Adaptive signalling depends on readiness—the capacity to generate, receive, terminate, and integrate a signal when the system requires it. This makes the prior state of the cellular environment biologically important. Demand may initiate the response, but capacity constrains how that demand can be met.

Context-dependent

A signalling event does not have one universal meaning. The same receptor can couple to different intracellular pathways depending on cell type, subcellular location, interacting proteins, membrane domain, ligand concentration, duration of exposure, and prior activation history. Biological response is therefore conditional. It emerges from the relationship between signal and context.

This is particularly important for a regulatory network associated with homeostasis. Homeostasis is not a fixed point. It is the active maintenance of function under changing conditions. A system that supports this process must be capable of different responses in different contexts. The ECS is therefore better understood as part of a flexible regulatory architecture than as a linear pathway with a single predictable output.

Tightly connected to membranes

The membrane is where the substrate-driven argument becomes spatially concrete. Endocannabinoid precursors, synthetic and degradative enzymes, cannabinoid receptors, and interacting signalling machinery are organized within or near lipid membranes. Membrane composition influences physical properties such as order, thickness, curvature, fluidity, domain formation, and protein mobility. These properties can affect how signalling components encounter one another and how receptors are positioned within the cellular interface. [2–7]

It would be an overstatement to infer receptor behaviour from a blood fatty-acid profile. Whole blood is not a direct measurement of a specific neuronal, immune, hepatic, or mitochondrial membrane.

Different tissues regulate their lipid composition differently, and bulk composition cannot reveal local membrane domains or receptor microenvironments. Yet the membrane remains the correct conceptual center because it is the biological layer in which substrate state and signalling organization meet.

The substrate-driven ECS framework

The framework proposed here places four interacting layers around the cellular membrane. Environmental inputs shape substrate availability and metabolic demand. The lipid substrate environment provides the material context from which membrane organization and lipid-derived mediators emerge. The membrane organizes receptors, enzymes, and signalling partners. Downstream networks translate this context into responses that feed back into future biological demand.

This is not a simple chain in which fatty acids drive ECS activity and ECS activity determines physiology. It is a systems relationship. Each layer constrains and informs the others, and the direction of influence can change over time. Nutritional exposure may alter substrate availability; metabolic state may alter utilization; signalling may alter enzyme activity; physiological adaptation may change future demand. The membrane functions as an interface within this loop, not as one box in a linear pathway.

The practical consequence is a shift in what we try to measure. Receptor expression alone cannot describe signalling capacity. Ligand concentration alone cannot describe interpretation. A fatty-acid profile alone cannot describe membrane function. But each can contribute a layer to a more complete representation of the biological state in which adaptive signalling occurs.

> **Framework claim: The cellular environment shapes the context in which lipid-responsive signalling systems—including the ECS—operate.**
>
> **Boundary of inference: Fatty-acid composition does not directly measure ECS activity, receptor function, or physiological outcome.**

What this framework is designed to do

A useful framework should make a question more testable. The substrate-driven ECS perspective does this by identifying the missing measurements between exposure and outcome. If lipid composition is one measurable layer of cellular context, it can be studied longitudinally alongside direct or functional readouts: circulating or tissue endocannabinoids, enzyme activity, receptor localization, downstream signalling responses, metabolic physiology, autonomic regulation, sleep, inflammatory state, and resilience following perturbation.

The immediate objective is therefore not to claim that the observed fatty-acid transitions altered ECS function. It is to establish a disciplined starting point for the next experiment: did the substrate environment change in a coordinated way, and can future work determine whether such changes correspond with altered adaptive signalling capacity?

3. From fatty-acid biomarkers to biological state variables

A biomarker tells us about a measurement. A state variable helps describe the condition of a dynamic system. The difference is not semantic; it changes the scientific question we are able to ask.

The value—and the limit—of a single index

Fatty-acid analysis is usually read through individual values. The omega-3 index is the most familiar example. It compresses selected fatty-acid information into a clinically interpretable number and provides a practical way to compare measurements across people or across time. Such indices are useful because they simplify complexity without making measurement impossible.

But simplification always leaves information behind. Two profiles can share a similar omega-3 index while differing in EPA, DHA, DPA-n3, omega-6 fatty acids, monounsaturated fatty acids, saturated fatty acids, or very-long-chain components. They may therefore occupy different overall compositional configurations even when one familiar marker appears similar.

The limitation is not that the biomarker is wrong. It is that the biological system contains relationships the single value was never designed to represent. A marker answers the question for which it was constructed. A systems analysis begins when we ask what remains invisible around that answer.

> **The traditional question: What is the omega-3 index?**
>
> **The systems question: Where does this lipid composition sit within biological space?**

Composition as a configuration

A fatty-acid profile is compositional. Each measured value exists in relation to the others, and the pattern is constrained by the total composition being described. An increase in relative abundance for one component may occur alongside stability, decrease, or redistribution elsewhere. This is why isolated trajectories are informative but incomplete. The biological pattern lies not only in the direction of one line, but in the coordinated movement of the profile.

Treating the profile as a configuration preserves those relationships. Omega-3, omega-6, monounsaturated, saturated, and very-long-chain fatty acids can be viewed as contributing dimensions of a broader lipid landscape. The state at any time point is represented by the joint arrangement of those dimensions, not by a single dominant marker.

This does not imply that every fatty acid contributes equally or that a larger panel is automatically more meaningful. Variable selection, measurement stability, biological relevance, and missingness all matter. The value of the multidimensional view is that it can reveal coordinated structure while keeping the contribution of individual components available for interpretation.

What is a biological state variable?

In systems science, a state variable is a measurement—or set of measurements—that helps specify the condition of a system at a given time. It does not have to explain every mechanism. It must carry

information about the system's current configuration and support comparison with another configuration.

In this context, state variable refers to a measurable descriptor of system configuration rather than a validated physiological state classifier. Whole-blood fatty-acid composition may function as a state variable if the combined profile can distinguish longitudinal configurations, reveal stable and transitional periods, and support reproducible relationships with other biological measurements. The claim is not that the fatty-acid profile is the state of the organism. It is one candidate variable describing one layer of that state: the lipid substrate environment.

This distinction protects the framework from overreach. A state variable can be informative without being causal. Temperature describes the state of a physical system without explaining every molecular collision. In the same way, a lipid composition state may help locate a biological system without, by itself, identifying the mechanisms that produced the state or the functions that follow from it.

> **Biomarker: a selected measurement interpreted in relation to a defined biological or clinical question.**
>
> **State variable: a measurement or configuration used to describe where a dynamic system is at a particular time.**

Locating composition within biological space

The phrase biological space is a way of describing many measurements at once. Each fatty acid can be treated as one dimension. A time point then occupies a position defined by the combined values across those dimensions. Because direct visualization of eleven or more dimensions is impossible, dimensionality-reduction methods such as principal component analysis provide a lower-dimensional map of the dominant relationships in the dataset.

The position on that map is relative, not absolute. It shows how the measured configurations relate to one another within the available data. Distance suggests compositional dissimilarity; proximity suggests similarity. A trajectory connects time points and makes temporal movement visible. The map does not define a universal healthy region, nor does movement in a particular direction imply improvement.

This restraint is essential. Multidimensional analysis can make change look biologically dramatic because distance becomes visually intuitive. The figure is a representation of variance in the measured profile. Meaning must come from study design, replication, functional association, and biological interpretation—not from geometry alone.

Time turns a profile into a trajectory

A single profile has position. A longitudinal series has direction. Once measurements are repeated, the analysis can begin to distinguish phases: broad remodeling, relative stability, secondary transition, or return toward an earlier configuration. It becomes possible to ask not only whether values differ, but when change occurred, how large the transition was, which components contributed, and whether multiple lipid classes moved together.

This temporal dimension is central to biological adaptation. Adaptation is rarely instantaneous. Exposure is followed by incorporation, utilization, remodeling, feedback, and—sometimes—an emerging equilibrium. Different components can operate on different timescales. A stable interval may represent true biological stability, a plateau in the measured variables, or simply insufficient

temporal resolution. A transition may reflect a new input, delayed incorporation, changed demand, altered metabolism, or several influences at once.

Longitudinal data therefore provide a richer description but not automatic causal inference. Time orders observations; it does not isolate the reason for change. The strength of the present approach is its ability to reveal structured movement. The causes of that movement remain questions for controlled investigation.

Whole blood as a measurement window

Whole-blood fatty-acid profiling is used here as a practical measurement window into the circulating lipid environment. It captures fatty acids associated with plasma and blood-cell compartments and can reflect integrated exposure and metabolism over time. Repeated sampling makes coordinated longitudinal change measurable with a method that is accessible and scalable.

At the same time, whole blood should not be treated as a direct surrogate for every tissue membrane. Brain, immune, hepatic, adipose, muscle, and mitochondrial membranes have different compositions, turnover rates, and regulatory controls. Blood composition cannot reveal receptor localization, membrane microdomains, lipid asymmetry, or local enzymatic activity. The phrase measurement window is therefore intentional: the profile provides a view into one biological layer, not a complete image of cellular organization.

How the analytical figures operationalize the concept

The figures that follow translate the state-variable concept into a sequence of analytical questions. Figure 1 asks whether individual fatty-acid trajectories changed over time. Figure 2 asks whether the joint profile moved within multidimensional composition space. Figure 3 asks whether an independent clustering method recovers the same pattern of similarity between time points. Figure 4 asks how far each interval moved and which fatty acids contributed to that movement. Figure 5 asks whether the final transition can be understood as coordinated redistribution across lipid classes.

No single figure establishes a biological state. The argument comes from convergence across views. Trajectories preserve the original measurements. PCA summarizes correlated variation. Clustering tests relative similarity from another mathematical perspective. Transition vectors restore direction and component contribution. Lipid-class decomposition brings the result back to interpretable biology.

This progression is deliberately systems-oriented. It moves from parts to configuration, from configuration to movement, and from movement back to the parts that produced it. The result is not a diagnosis or a score. It is a map of longitudinal compositional change that can be paired with future functional measurements.

The testable proposition

The conceptual transition from biomarker to state variable becomes scientifically useful only if it generates testable predictions. If the fatty-acid profile reflects a meaningful layer of cellular context, then reproducible lipid-state transitions should correspond—at least in some settings—with changes in other biological domains. These might include endocannabinoid-related measurements, metabolic flexibility, autonomic regulation, inflammatory response, sleep physiology, membrane biophysics, or recovery following a defined perturbation.

The relevant question is not whether one ‘optimal’ lipid state exists. Different tissues, individuals, and physiological demands may require different configurations. The more useful question is

whether the system can be located, followed, and related to function across time. That is the promise of the state-variable framework: not a better snapshot, but a disciplined way to study biological movement.

> **Working proposition: Longitudinal fatty-acid composition may provide a measurable representation of changing lipid substrate state, suitable for integration with direct measures of signalling and physiology.**

4. Results

The longitudinal profile did not behave as a fixed nutritional signature. Across four measurements, individual fatty acids changed, the relationships among them reorganized, and the combined profile moved through compositionally different configurations.

The results are presented as a sequence of analytical views. The first preserves the trajectories of individual fatty acids. The second and third examine the configuration of the complete stable panel. The fourth resolves movement into direction, magnitude, and component contribution. The fifth returns to lipid classes to show how the final transition was distributed across the wider composition. Together, these views describe the same longitudinal system at different levels of resolution.

> **Results boundary: The analyses describe compositional movement. They do not identify the mechanism that produced it or establish physiological benefit.**

4.1 Longitudinal fatty-acid trajectories reveal dynamic composition states

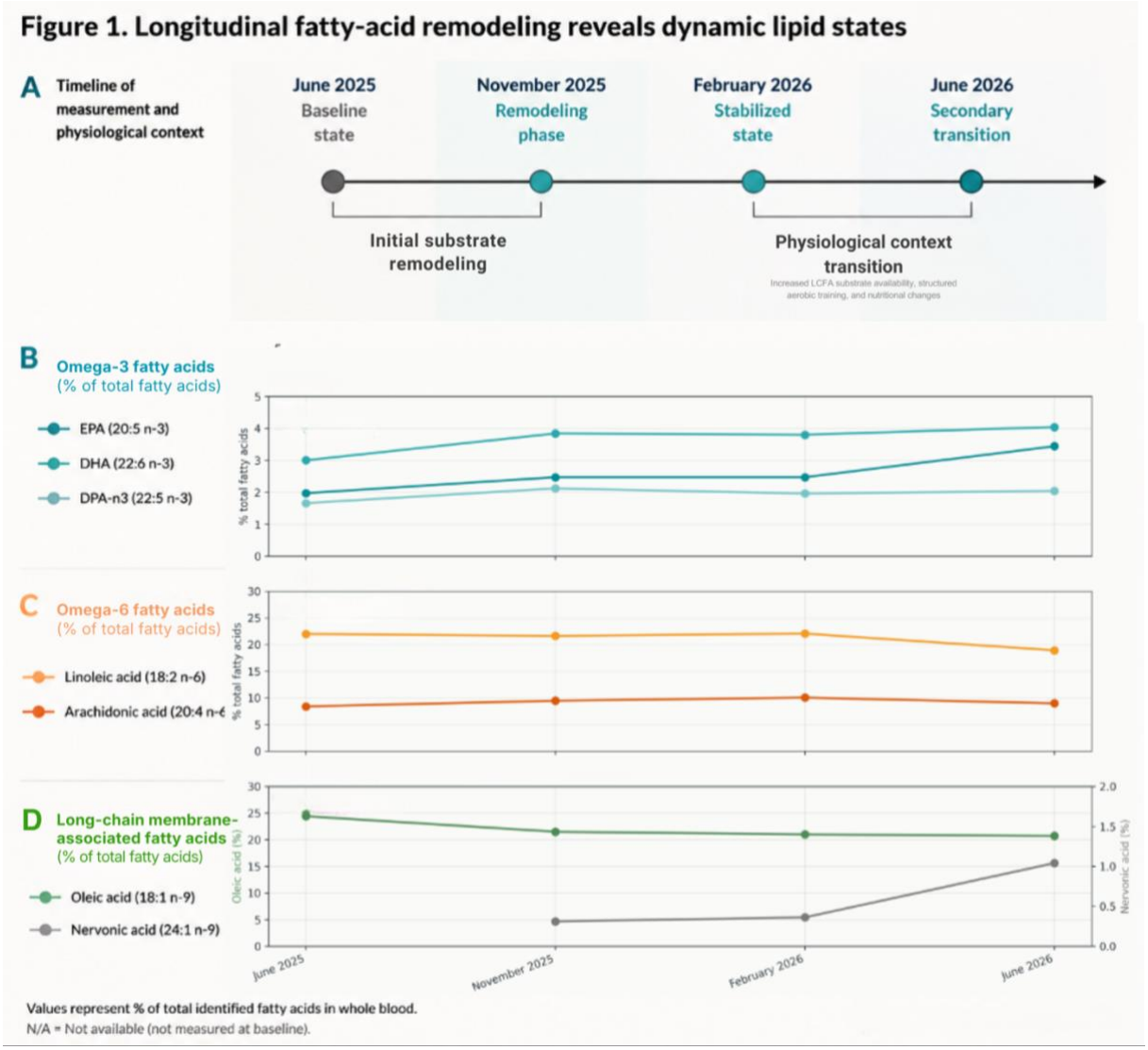


Figure 1. Longitudinal fatty-acid composition trajectories reveal dynamic lipid states over time. (A) Timeline of longitudinal whole-blood fatty-acid measurements and associated physiological context. Four measurement time points were analyzed: June 2025, November 2025, February 2026, and June 2026. The longitudinal period included changes in nutritional fatty-acid exposure, structured aerobic training, and other physiological factors that may influence lipid composition. **(B)** Temporal trajectories of measured omega-3 fatty acids. EPA, DHA, and DPA-n3 demonstrated progressive changes across the observation period, with the largest increase in EPA observed between February and June 2026. **(C)** Temporal trajectories of omega-6 fatty acids. Linoleic acid and arachidonic acid remained relatively stable during the initial observation period, followed by a decrease in both measures between February and June 2026. **(D)** Temporal trajectories of membrane-associated long-chain fatty acids. Oleic acid showed a gradual decrease across the measurement period, while nervonic acid, a very-long-chain monounsaturated fatty acid (VLC-MUFA), increased between November 2025 and June 2026. Nervonic acid was not measured at the June 2025 profiling. Values represent percentage abundance of identified fatty acids in whole blood. Changes describe longitudinal compositional trajectories and do not establish causality.

Figure 1 begins with the measured values themselves. Four whole-blood fatty-acid profiles—June 2025, November 2025, February 2026, and June 2026—provide a longitudinal view of the

composition across approximately one year. Read separately, each fatty acid has its own trajectory. Read together, the trajectories show that the lipid profile was not static.

The omega-3 series changed across the observation period. EPA, DHA, and DPA-n3 each contributed to the evolving profile, with the largest increase in EPA occurring between February and June 2026. The result is not simply a higher or lower value at the final measurement. It is a time-resolved pattern in which the scale and timing of change differed among related fatty acids.

The omega-6 series followed a different pattern. Linoleic acid and arachidonic acid were relatively stable across the earlier measurements and then decreased between February and June 2026. This later redistribution occurred during the same interval in which the omega-3 profile changed most clearly. The paired observation is descriptive: it establishes concurrent movement within the measured composition, not a direct exchange mechanism between the two classes.

The monounsaturated and very-long-chain measurements added a further dimension. Oleic acid declined gradually across the observation period, whereas nervonic acid increased from November 2025 to June 2026. Because nervonic acid was not measured in June 2025, its trajectory cannot be compared across all four time points and is treated separately where full-panel comparability is required.

Several features therefore changed at once: omega-3 composition shifted, omega-6 composition redistributed, and changes involving MUFA and VLC-MUFA became visible. The pattern cannot be reduced to one index or one fatty acid. At the level of direct observation, the central result is temporal movement. The fatty-acid landscape demonstrated change over time rather than behaving as a fixed nutritional signature.

4.2 Fatty-acid composition moves through multidimensional space

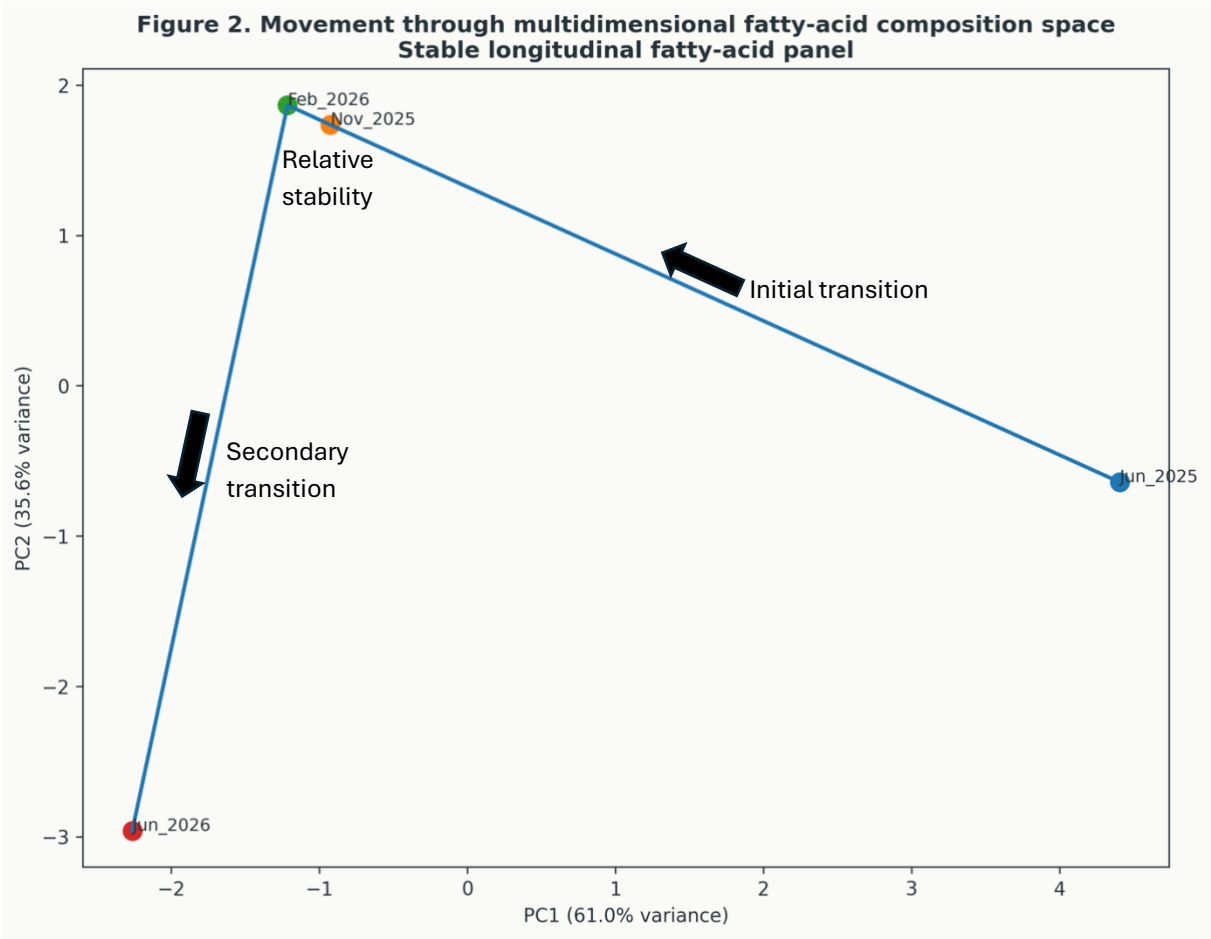


Figure 2. Longitudinal movement through multidimensional fatty-acid composition space. Principal component analysis (PCA) was performed using the stable 11-fatty-acid longitudinal panel measured at all four time points. Each point represents the overall fatty-acid composition state at an individual measurement time point after z-score transformation of fatty-acid abundances. The trajectory demonstrates movement from June 2025 toward an intermediate configuration observed in November 2025 and February 2026, followed by a further shift into a distinct compositional region by June 2026. PC1 and PC2 explain 61% and 35.6% of total variance, respectively. The PCA visualization describes changes in multivariate fatty-acid composition space and does not imply biological improvement or a specific physiological outcome.

A biological state is rarely represented by one molecule. The relevant information often lies in the relationships among molecules: which components rise together, which move in opposing directions, which remain stable, and whether the combined pattern at one time resembles or differs from the pattern at another. Figure 2 applies principal component analysis (PCA) to visualize these relationships within the stable 11-fatty-acid panel measured at every time point.

After standardization, each time point is represented by a single position derived from the complete panel. The first two principal components explain 61.0% and 35.6% of the variance, respectively—96.6% in combination—allowing nearly all of the variation in this four-time-point dataset to be displayed in two dimensions. The map therefore condenses the dominant structure of the measured profile without replacing the individual trajectories from which it was derived.

The June 2025 profile occupies one region of the composition space. November 2025 and February 2026 are positioned more closely, indicating greater similarity in their standardized multivariate profiles. By June 2026, the trajectory extends into another region, consistent with a further reorganization of the joint composition. Time has converted four profiles into a path: an initial transition, an interval of comparatively limited movement, and a later transition into a more distinct configuration within this dataset.

PCA does not assign biological value to that path. Its axes represent directions of variance, not scales of health, function, or adaptation. The geometry shows relative similarity and difference among the measured configurations; it does not reveal why the movement occurred or what physiological consequence followed.

> **Interpretive principle: The trajectory does not represent improvement or deterioration. It represents movement between compositionally different lipid configurations.**

4.3 Independent clustering supports compositionally distinct configurations

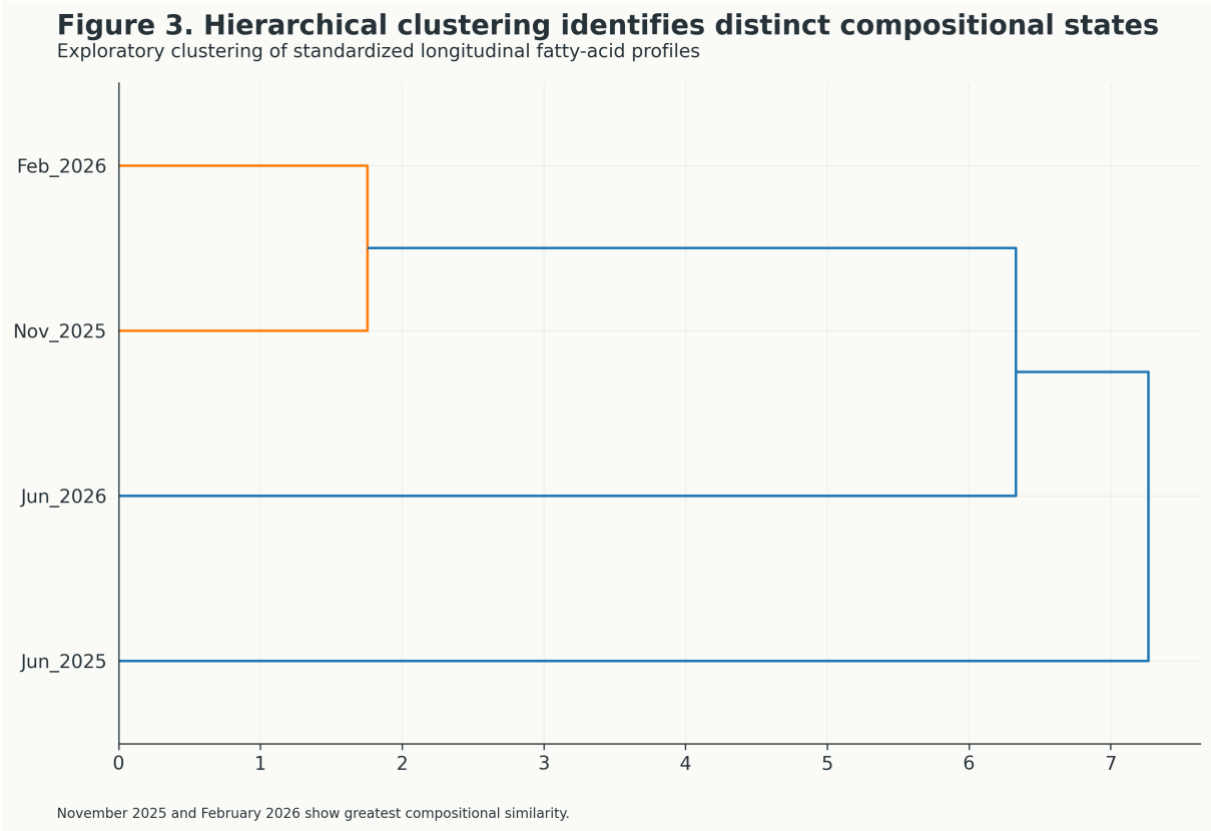


Figure 3. Hierarchical clustering reveals longitudinal relationships between longitudinal fatty-acid composition states. Hierarchical clustering was performed on standardized abundances from the stable 11-fatty-acid panel measured at all four longitudinal time points. Clustering was performed using Euclidean distance and Ward linkage. The analysis demonstrates the relative similarity between measured lipid composition states. November 2025 and February 2026 displayed the closest compositional relationship, whereas June 2025 and June 2026 occupied more distant positions within the longitudinal dataset. This analysis provides an orthogonal visualization of compositional relationships identified by PCA and does not represent classification into discrete biological states.

PCA shows movement by projecting the dominant variance onto a small number of axes. Hierarchical clustering asks a complementary question: when the same standardized fatty-acid profiles are compared through another mathematical framework, do the same relationships among time points remain visible? Figure 3 addresses this through Euclidean distance and Ward linkage.

The clustering result places November 2025 and February 2026 in the closest relationship. June 2025 and June 2026 are more distant within the longitudinal set. This structure is consistent with the PCA trajectory: the two intermediate measurements share the greatest compositional similarity, while the first and final measurements occupy more separated positions.

The value of this agreement is methodological rather than classificatory. PCA and clustering organize the data differently, yet both identify the relative proximity of the intermediate profiles and the greater separation of the endpoints. The convergence supports the presence of compositionally distinct configurations in the measured series.

It does not establish discrete biological states. Four longitudinal observations from one series cannot define universal classes, thresholds, or phenotypes. The dendrogram is best read as a map of relative similarity within this dataset. Its contribution is to show that the pattern visible in PCA is not dependent on a single mode of visualization.

4.4 Direction and magnitude of lipid-state transitions

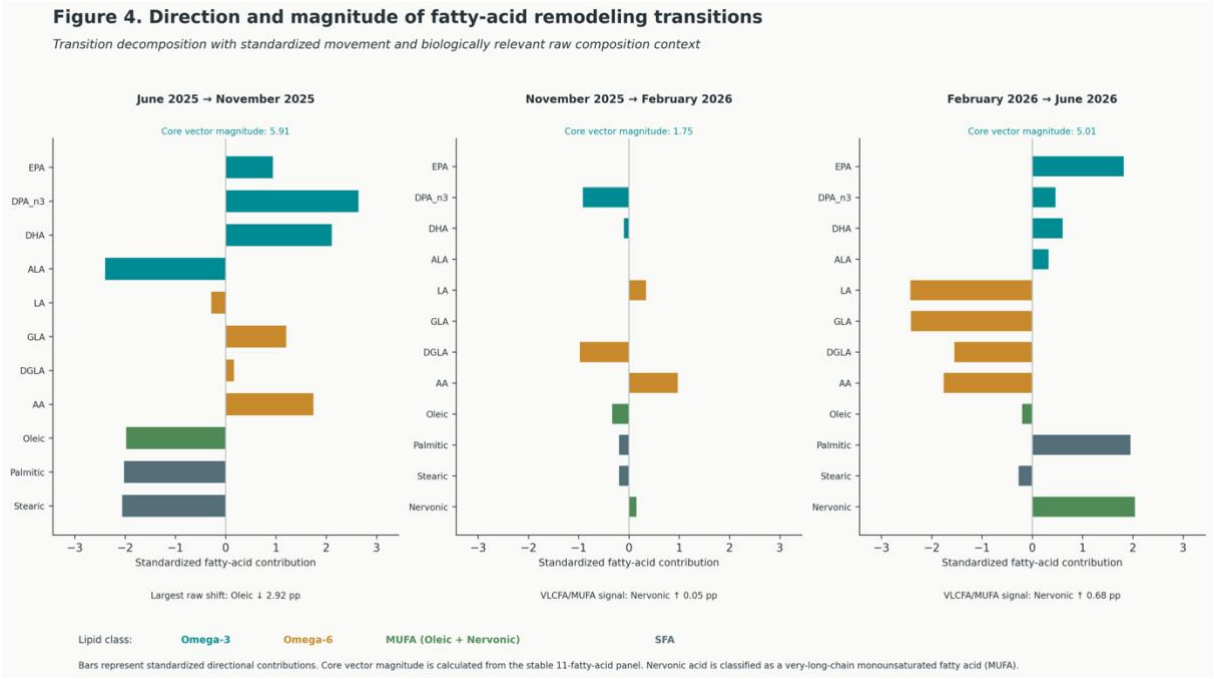


Figure 4. Direction and magnitude of fatty-acid composition transitions across longitudinal intervals. Transition vectors were calculated for each consecutive measurement interval to visualize the direction and relative contribution of individual fatty acids to longitudinal movement within fatty-acid composition space. The stable 11-fatty-acid panel was used to calculate comparable transition magnitudes across all intervals. Extended transitions including November 2025 to February 2026 and February 2026 to June 2026 additionally incorporate nervonic acid, which was only available from the extended measurement panel. The transition from June 2025 to November 2025 showed broad redistribution across multiple fatty acids. November 2025 to February 2026 demonstrated comparatively limited movement. The February 2026 to June 2026 transition showed coordinated changes involving increased omega-3 fatty acids, decreased omega-6 fatty acids, and additional VLC-MUFA changes including nervonic acid. Bars represent standardized directional contributions and should be interpreted as relative drivers of compositional movement rather than absolute concentration changes.

Once the profile is treated as a moving configuration, change can be examined with greater precision. A transition is not only a question of how far the system moved. It also has direction. Individual components can contribute with different signs and different magnitudes, and their combined pattern can reveal whether movement was narrow or distributed across the profile.

Figure 4 resolves each consecutive interval into standardized transition vectors. The June-to-November 2025 interval shows broad redistribution across multiple fatty acids. The November 2025-to-February 2026 interval shows comparatively limited movement, consistent with the proximity of those time points in both PCA and clustering. The February-to-June 2026 interval again shows broader movement, now involving increased omega-3 fatty acids, decreased omega-6 fatty acids, and additional change in the VLC-MUFA component represented by nervonic acid.

These vectors restore information that is compressed in a two-dimensional state-space map. Two intervals could, in principle, have a similar overall magnitude while being produced by different combinations of fatty acids. Conversely, several small component changes can align to produce a coordinated transition. Directional decomposition therefore connects the position of the system back to the measured variables that moved it.

The distinction between the stable and extended panels remains important. Comparable transition magnitudes across all intervals are calculated from the 11 fatty acids available at every time point. Nervonic acid can be included only in the later extended transitions because no June 2025 value was available. This preserves comparability while allowing the later VLC-MUFA signal to remain visible.

At this level, the analysis begins to take on a systems character. It asks not merely whether one marker changed, but how the configuration reorganized: how far it moved, in which direction, which components contributed, and whether those contributions formed a coordinated pattern. The vectors still do not explain mechanism. They provide a more complete description of movement within the lipid composition system.

4.5 Lipid-class redistribution reveals coordinated compositional change

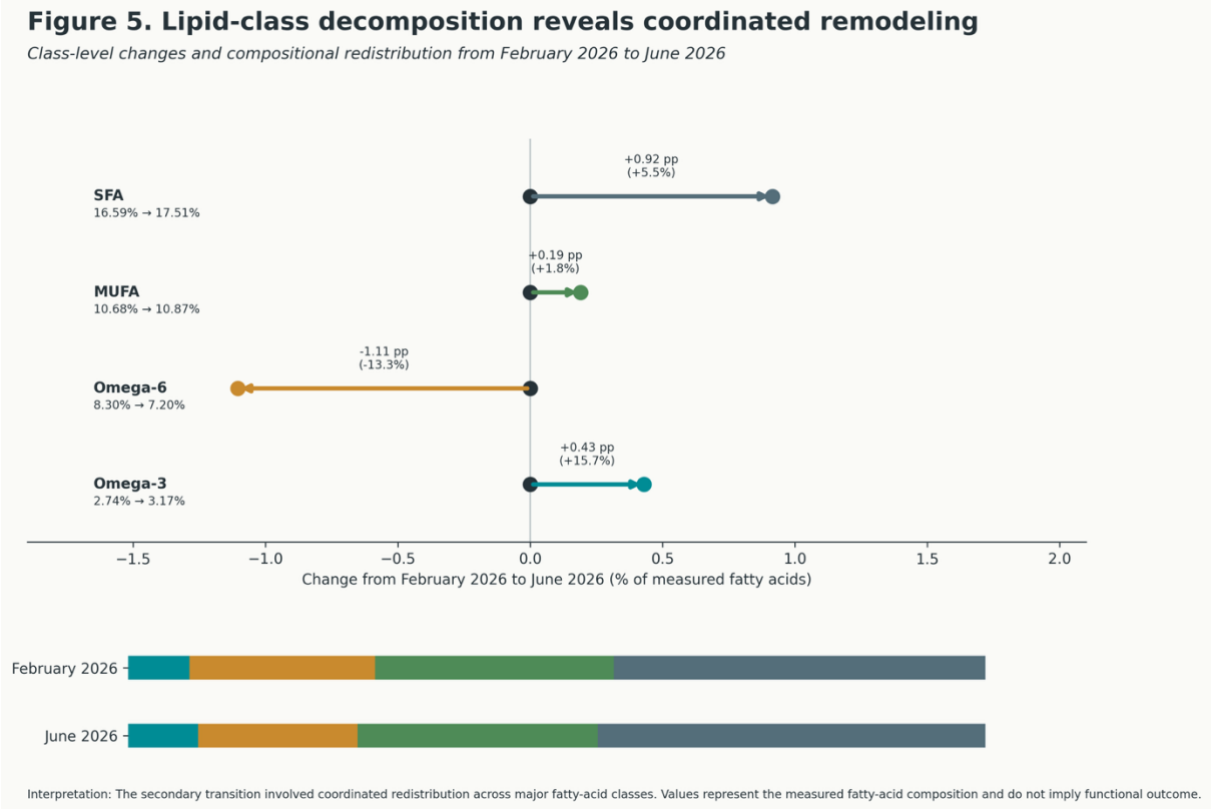


Figure 5. Lipid-class decomposition highlights coordinated redistribution during the February 2026 to June 2026 transition. Fatty acids were grouped into major lipid classes to examine class-level compositional changes during the final longitudinal transition. Omega-3 fatty acids were calculated from EPA, DPA-n3, and DHA; omega-6 fatty acids from linoleic acid, DGLA, and arachidonic acid; MUFA from oleic acid and nervonic acid where available; and saturated fatty acids from palmitic and stearic acid. Between February and June 2026, the lipid composition demonstrated increased relative omega-3 contribution, decreased relative omega-6 contribution, and redistribution across monounsaturated and saturated fatty-acid classes. The stacked composition inset illustrates the relative contribution of each lipid class before and after the transition. These measurements describe changes in fatty-acid composition and do not determine the biological mechanisms responsible for the observed redistribution.

Figure 5 brings the analysis back from individual transition vectors to biologically recognizable lipid classes. The February-to-June 2026 interval was selected because it represented the clearest later movement in the preceding analyses. Grouping the measured fatty acids as omega-3, omega-6, MUFA, and saturated fatty acids shows how that transition was distributed across the composition.

The final interval included an increased relative omega-3 contribution, a decreased relative omega-6 contribution, and redistribution within the monounsaturated and saturated components. The stacked composition view makes the reciprocal nature of compositional data visible: the profile changed through altered relationships among classes, not through the isolated movement of a single constituent.

This class-level view does not supersede the fatty-acid trajectories. It synthesizes them. EPA, DHA, and DPA-n3 contribute to the omega-3 class; linoleic acid, DGLA, and arachidonic acid contribute to omega-6; oleic and available nervonic acid contribute to MUFA; and palmitic and stearic acid contribute to the saturated class. The aggregate pattern is therefore traceable to measured components rather than being an abstract score.

Across Figures 1–5, the same conclusion is reached at progressively different scales. Individual trajectories changed. The multivariate profile moved. Independent clustering preserved the relative relationships among time points. Transition vectors identified direction and contribution. Lipid-class decomposition showed that the later transition involved coordinated redistribution across the wider profile.

The transition was not driven by one fatty acid. It reflected a reorganization across lipid classes, supporting the concept of a changing lipid environment. Whether that changing environment altered membrane organization, endocannabinoid signalling capacity, or physiological adaptation remains outside the present measurements and defines the next level of investigation.

4.6 Analytical overview

The analyses were designed to examine longitudinal fatty-acid composition at increasing levels of biological organization. Individual fatty-acid trajectories were first evaluated to establish the measured changes over time. Multivariate approaches were then applied to assess whether the combined fatty-acid profile occupied different compositional configurations across measurement points. Principal component analysis (PCA) was used to visualize movement within multidimensional fatty-acid composition space, while hierarchical clustering provided an independent assessment of similarity between longitudinal profiles. Transition-vector analysis subsequently examined the direction, magnitude, and component contribution of compositional movement between consecutive time points. Finally, lipid-class decomposition was used to summarize how the observed redistribution was distributed across broader fatty-acid categories.

All primary longitudinal analyses were based on the stable 11-fatty-acid panel measured across all four time points. Nervonic acid, which was only available from November 2025 onward, was retained as an exploratory extended-panel observation and was not used to define the primary longitudinal composition space. Detailed dataset construction, variable selection, preprocessing, and analytical methods are provided in Appendix 2.

5. Discussion: from fatty acids to cellular context

The significance of these observations lies less in any single fatty-acid value than in the possibility that the combined profile can help describe the biological environment in which signalling takes place.

A wider interpretation of fatty-acid composition

Fatty acids have traditionally been interpreted through several well-established lenses. They can serve as markers of dietary exposure, particularly when intake is expected to influence circulating or cellular abundance. They can be examined in relation to cardiovascular risk, inflammatory balance, or metabolic health. These interpretations remain valuable and should not be displaced by a systems framework.

The present analysis proposes an additional question. Beyond what fatty acids reveal about exposure or association with a defined outcome, might the composition also help describe the substrate environment in which cellular signalling occurs? This is a different level of interpretation. It does not turn a whole-blood profile into a direct membrane measurement. It treats the profile as a measurable window into one layer of the biochemical context from which membranes, lipid mediators, and signalling networks are organized.

That distinction matters because cells do not interpret signals in a vacuum. Receptors, enzymes, transporters, and downstream effectors operate within membranes and intracellular compartments whose organization depends on available substrates, metabolic activity, prior state, and local demand. A change in fatty-acid composition does not dictate the resulting signal. It may, however, indicate that the material setting in which signalling occurs has become compositionally different. [2–7,11,12]

The operating environment matters

A useful analogy is a computer with unchanged hardware operating under different system conditions. The processor, memory, and circuitry may be physically present in both cases, yet performance depends on the operating environment: available resources, active processes, system configuration, background demand, and the way information is routed. Hardware alone does not determine the behaviour of the complete system.

A receptor can be considered in a similar way. Its molecular identity may be unchanged, but its signalling behaviour depends on where it is located, which membrane domain contains it, which ligands and precursors are available, which enzymes and interacting proteins are nearby, and which downstream pathways are ready to respond. The receptor is the hardware only in a limited analogy. The membrane and cellular environment help define the operating conditions under which that hardware functions. [6,7]

The analogy is not intended to make biology mechanistic or programmable. Living systems remodel themselves, respond to feedback, and change the conditions of their own future responses. Its purpose is to make one principle intuitive: the existence of a receptor does not, by itself, describe signalling capacity. Context is not background noise around the signal. Context participates in determining what the signal can mean.

> **Central proposition: Fatty-acid composition may help describe the substrate environment in which cellular signalling occurs, without directly measuring the signalling event itself.**

Reading the longitudinal pattern as a system

The figure set supports this proposition at a descriptive level. Figure 1 establishes that the measured profile changed across time. Figure 2 shows that the joint composition moved within a multidimensional space. Figure 3 demonstrates that the relative relationships among time points are recovered through an independent clustering approach. Figure 4 resolves the intervals into direction, magnitude, and component contribution. Figure 5 shows that the later transition was distributed across lipid classes rather than driven by one isolated fatty acid.

The importance of this convergence is not that it proves a new biological state. It shows that the composition contains structured longitudinal information. The intermediate measurements were relatively similar, whereas the baseline and final measurements were more separated. The later transition involved coordinated increases, decreases, and redistribution across the measured panel. In systems terms, the profile changed configuration.

The data therefore support a disciplined shift in emphasis—from asking only what happened to a selected biomarker toward asking where the complete lipid composition was positioned, how it moved, and which relationships produced that movement. This is the practical foundation of a state-variable approach. The profile becomes useful not because it replaces established markers, but because it preserves relationships that individual markers necessarily compress.

From association toward testable context

The present work remains hypothesis-generating. Whole-blood fatty-acid composition cannot specify the lipid organization of a particular neuronal, immune, hepatic, muscle, adipose, or mitochondrial membrane. Nor can the observed transitions identify changes in receptor localization, endocannabinoid synthesis, enzyme activity, or downstream signalling. The findings become scientifically valuable by making those missing relationships explicit.

If composition represents one layer of cellular context, future studies can ask whether transitions in that layer correspond with changes in direct or functional measurements. The relevant design is no longer a single fatty-acid test paired with a distant endpoint. It is a synchronized longitudinal model in which substrate state, signalling measurements, physiological context, and functional response are followed together.

This is where ECS Restore becomes differentiated. Its opportunity is not to rename fatty-acid profiling as an ECS test. It is to build a framework in which lipid composition is one measurable layer of the adaptive environment, deliberately integrated with measurements capable of testing signalling and physiology more directly.

6. Implications for ECS biology

The endocannabinoid system provides a compelling test case for substrate-driven biology because its signals are lipid-derived, produced on demand, organized around membranes, and interpreted through context-dependent pathways. [6–10]

What the present data do—and do not—say

The present data do not show ECS activation. No endocannabinoid ligand, synthetic or degradative enzyme, cannabinoid receptor, receptor-localization pattern, or downstream ECS response was measured. It would therefore be inappropriate to interpret movement in fatty-acid composition space as increased or decreased ECS activity.

The observations instead identify a changing lipid substrate environment. This matters to ECS biology because endogenous cannabinoid signalling is generated and received within lipid-rich cellular contexts. Precursor availability, membrane organization, enzymatic control, receptor localization, and downstream coupling all contribute to signalling capacity. A compositional transition may be relevant to that capacity, but relevance is a hypothesis to be tested rather than a conclusion contained in the present dataset. [6–10]

> **Interpretive boundary: The observations motivate investigation into whether lipid-state transitions correspond with changes in adaptive signalling capacity.**

From receptor activation to signalling capacity

Receptor-centric experiments remain essential when the objective is to determine ligand binding, potency, selectivity, or pathway activation. A substrate-driven model asks a complementary question: under what cellular conditions does that receptor operate? This expands the unit of analysis from a receptor-ligand pair toward a context-sensitive signalling system.

Adaptive signalling capacity is a useful concept for this expansion. It refers not to continuous activation, but to the ability of a system to generate, receive, regulate, terminate, and integrate signals when biological demand arises. Such capacity is expected to depend on several layers at once: substrate availability, enzymatic competence, membrane organization, receptor positioning, cellular energy state, and the readiness of downstream networks.

The ECS is especially relevant because it participates in regulation rather than a single fixed output. Its role varies across neural, immune, metabolic, gastrointestinal, and other physiological contexts. A signal that is adaptive in one tissue, time, or demand state may not have the same meaning in another. For this reason, a future ECS-state framework should avoid interpreting more signalling as inherently better. The central question is whether regulation is appropriately matched to context.

A measurement architecture for future ECS studies

The framework suggests a layered measurement strategy. Fatty-acid composition can characterize part of the substrate environment. Circulating or tissue endocannabinoids can describe selected mediator levels. Enzyme activity or expression can inform synthetic and degradative capacity. Receptor localization, availability, or functional challenge tests can examine signal reception. Physiological measurements can show whether these layers correspond with regulation at the level of the organism.

The value would come from temporal alignment. If each layer is measured at unrelated times, dynamic relationships may be missed. Repeated, synchronized sampling could reveal whether a lipid-state transition precedes, accompanies, or follows a change in signalling or function. Perturbation studies could then test whether the system responds differently depending on its starting configuration.

7. Limitations

The credibility of a systems framework depends on being explicit about what the present observations cannot establish.

A single longitudinal individual

The dataset represents one individual measured at four time points. It cannot establish population distributions, reproducibility across individuals, sex- or age-related differences, clinical reference ranges, or generalizable lipid-state classes. The observed configurations are relative positions within this longitudinal series, not validated phenotypes. Replication in larger and more diverse cohorts is required before the analytical patterns can be generalized.

Observational design and simultaneous change

The observation period included multiple concurrent influences, including changes in nutritional fatty-acid exposure, structured aerobic training, and other physiological factors. These influences were not isolated experimentally. The temporal relationship between an exposure and a measured transition therefore cannot identify which factor produced the change, whether several factors interacted, or whether unmeasured influences contributed.

No randomized intervention or control condition

There was no randomized intervention, comparator, washout period, or controlled challenge. The analysis cannot distinguish intervention effects from natural variability, measurement variability, regression toward a prior value, seasonal effects, or time-dependent physiological change. Longitudinal ordering shows when differences were observed; it does not establish causality.

Measurement scope

Whole-blood fatty-acid abundance is a composite measurement across plasma and blood-cell compartments. It is not a direct assay of a defined tissue membrane and cannot resolve membrane leaflets, lipid domains, organelle-specific composition, local turnover, or the microenvironment surrounding a particular receptor. Tissue-specific regulation may diverge substantially from the circulating profile.

Nervonic acid was unavailable at the June 2025 profiling. It was therefore excluded from the stable 11-fatty-acid PCA and from directly comparable core transition magnitudes. Its later trajectory and extended-panel contribution are exploratory and should not be interpreted as a complete four-time-point pattern.

Analytical scope

PCA and hierarchical clustering are descriptive tools applied here to a very small number of time points. The high proportion of variance displayed by the first two principal components reflects the structure of this limited dataset and should not be assumed to generalize. Clustering demonstrates relative similarity among the four observed profiles; it does not validate discrete biological categories.

Because fatty-acid measurements are compositional, relative increases and decreases are interdependent. Standardization facilitates multivariate comparison but changes the scale of

interpretation. Transition-vector contributions therefore describe standardized direction and relative influence, not absolute biological effect size.

No direct ECS or functional measurement

Fatty-acid composition does not directly measure ECS activity. The study did not quantify endocannabinoids, related lipid mediators, ECS enzymes, cannabinoid receptor activity or localization, downstream signalling, membrane biophysics, or a synchronized functional response. The data consequently cannot determine whether the observed lipid-state transitions changed adaptive signalling capacity or physiological outcome.

> **Appropriate conclusion: The dataset demonstrates structured longitudinal compositional change and supports a testable framework. It does not establish mechanism, clinical benefit, ECS activation, or generalizable biological states.**

8. Future direction: from static biomarkers to dynamic biological-state monitoring

The next step is not another isolated biomarker. It is a dynamic view of adaptive capacity built from synchronized measurements across biological layers.

The ECS Restore platform

ECS Restore is proposed as a broader translational platform for studying how substrate environment, signalling context, and physiology change together over time. The platform is not defined by one assay and should not be presented as a test of ECS activation. Its purpose is to organize complementary measurements into a longitudinal representation of biological context and response.

Fatty-acid composition provides the initial demonstration because it is measurable, biologically connected to membranes, and capable of revealing coordinated transitions. Its role within the platform is foundational but incomplete. The profile locates one layer of the system. Additional layers are required to determine whether a compositional state corresponds with signalling readiness, physiological demand, or functional adaptation.

An integrated measurement model

Future ECS Restore studies should integrate four measurement domains. Longitudinal fatty-acid composition can describe the changing lipid substrate state. Sleep physiology can capture timing, continuity, architecture, and recovery during a major regulatory state. Autonomic biomarkers can characterize dynamic balance and responsiveness through measures such as heart-rate patterns and variability. Metabolic measurements can place these signals within energy availability, utilization, and flexibility.

A fifth domain—functional signalling readouts—is essential for testing the framework itself. Depending on the study context, these may include endocannabinoid and related lipid-mediator profiles, enzyme activity, receptor localization or responsiveness, cellular challenge assays, inflammatory responses, or other measures that directly interrogate signalling function. Without this

layer, the platform can describe context but cannot determine how that context changes signalling capacity.

The measurements should be synchronized and repeated. A useful system must be able to distinguish baseline configuration, perturbation, transition, response, recovery, and possible stabilization. The timing of sampling should reflect the expected timescale of each layer: rapid autonomic and sleep changes, intermediate metabolic responses, and potentially slower compositional remodeling.

From reference ranges to trajectories

Conventional biomarkers are commonly interpreted by comparing one value with a reference range. Dynamic monitoring adds a different form of information: the individual trajectory. It asks where the system started, how it responded to a defined change, whether multiple layers moved together, and whether the system recovered or stabilized in a new configuration.

This does not eliminate the need for population references. It creates a second axis of interpretation. Population data can describe expected ranges and variation; longitudinal data can describe within-person movement and responsiveness. Over time, larger cohorts may show whether recurring trajectories or transition patterns correspond with defined physiological contexts.

The distinction is particularly important for adaptive biology. Capacity may be revealed most clearly during change. A system that appears similar at rest may respond differently to sleep disruption, exercise, dietary change, inflammatory challenge, or metabolic demand. Monitoring the path through perturbation and recovery may therefore be more informative than measuring the resting point alone.

A staged research program

The first stage is analytical replication: repeat fatty-acid trajectory, PCA, clustering, and transition analyses in larger longitudinal cohorts with standardized sampling and explicit assessment of measurement reproducibility. The objective is to determine which compositional relationships are stable, which are individual, and which recur across defined exposures or physiological contexts.

The second stage is multimodal association: synchronize lipid composition with sleep, autonomic, metabolic, inflammatory, and direct signalling measurements. The objective is to test whether transitions align across layers and to identify the time lags that separate exposure, substrate remodeling, signalling response, and physiological adaptation.

The third stage is controlled perturbation: introduce defined nutritional, exercise, sleep, or metabolic challenges with comparator conditions. The objective is to determine whether starting configuration predicts response, whether coordinated changes are reproducible, and whether specific transitions correspond with altered functional signalling capacity.

The fourth stage is adaptive-state modelling: integrate repeated measurements into interpretable models that can distinguish current configuration, direction of movement, response to perturbation, and recovery. Such models should remain biologically transparent. Their value will depend not only on prediction, but on showing which measurement layers contribute and where uncertainty remains.

The larger objective

The ambition of ECS Restore is not to produce another score detached from biology. It is to develop a dynamic view of adaptive capacity: a way to observe how the biochemical environment, signalling machinery, and physiological response relate across time.

The membrane remains the conceptual anchor. It is where environmental inputs meet cellular organization, where lipid-derived signals are generated, where receptors are positioned, and where changing substrate conditions may influence the context of response. Fatty-acid composition offers one measurable path into that interface. Sleep, autonomic, metabolic, and functional signalling measurements extend the view outward and inward.

The present White Paper establishes the first layer of that program. It shows that longitudinal fatty-acid composition can be treated as a moving configuration rather than a static signature. The next scientific task is to determine whether that movement corresponds with changes in adaptive signalling and physiology. If it does, the result will not be one more biomarker. It will be a more integrated way of observing biological state, transition, and capacity.

Data availability

The original whole-blood fatty-acid reports underlying this White Paper are reproduced in Appendix 1. The harmonized analytical dataset, variable definitions, and methodological procedures used to generate the longitudinal analyses are provided in Appendix 2. These materials are included to allow direct inspection of the relationship between the source measurements and the derived figures.

Conflict of interest

The author is the founder of ECS Restore and has intellectual-property interests related to technologies and approaches for investigating and supporting endocannabinoid-system function. The present White Paper includes longitudinal observations from the author and should therefore be interpreted as hypothesis-generating rather than independent validation. No commercial product efficacy claims are made.

Author contributions

Stefan Broselid conceived the scientific framework and study approach; collected, curated, and interpreted the longitudinal data; developed the analytical framework; prepared the figures; and wrote and revised the manuscript.

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Appendix 1. Longitudinal whole-blood fatty-acid profiles

The following appendix provides the original fatty-acid profile reports underlying the longitudinal analysis presented in this White Paper. Four whole-blood fatty-acid measurements were included: June 2025, November 2025, February 2026, and June 2026. The original reports are reproduced to preserve transparency regarding the source data used for subsequent trajectory, multivariate, clustering, transition, and lipid-class analyses.

ZINZINO
BALANCETEST

Detaljer kring fettsyror som mäts

Datum	11.06.2025
Land	Sweden
Kön	Man
Ålder	39

Mättat fett	Målintervall, %	Ditt värde	Avvikelse (i %)
Palmitinsyra (PA) C16:0	23.12 - 25.05	22.90%	-1%
Stearinsyra, C18:0	12.51 - 13.77	13.80%	0.2%

Omega-9	Målintervall, %	Ditt värde	Avvikelse (i %)
Oljesyra (OA) C18:1	20.93 - 23.39	24.40%	4.3%

Omega-6	Målintervall, %	Ditt värde	Avvikelse (i %)
Linolsyra (LA) C18:2	18.44 - 21.26	22.00%	3.5%
Gamma-linolensyra (GLA), C18:3	0.11 - 0.22	0.08%	-27.3%
Dihomo-gamma-linolensyra (DGLA) C20:3	0.91 - 1.16	1.09%	0%
Arakidonsyra (AA) C20:4	6.50 - 9.50	8.40%	0%

Omega-3	Målintervall, %	Ditt värde	Avvikelse (i %)
Alfa-linolsyra (ALA), C18:3	0.38 - 0.63	0.64%	1.6%
Eikosapentaensyra (EPA) C20:5	3.33 - 5.02	1.97%	-40.8%
Dokosapentaensyra (DPA) C22:5	1.95 - 2.36	1.66%	-14.9%
Dokosahexaensyra (DHA) C22:6	4.23 - 4.95	3.00%	-29.1%

COMPLETE FATTY ACIDS REPORT

Name: Stefan Broselid
DOB: 1985-07-04
Account: Customer

Sample ID: EUQIRYU4
Result date: 2025-11-14

UK | Europe | Australia
info@fattyacidlabs.com
results.fattyacidlabs.com

Type		Fatty Acids	Whole Blood Level	Model Person Range	SD Value
Polyunsaturated Fats (PUFAs)	Omega-3	**Omega-3 Fatty Acids**	**8.69%**		
		Omega3 Index	8.5%	8.00 - 12.00%	
		Alpha-Linolenic (C18:3n3)	0.27%	0.08 - 0.68%	0.3
		Eicosapentaenoic (EPA, C20:5n3)	2.47%	2.93 - 4.21%	0.64
		Docosapentaenoic-n3 (C22:5n3)	2.12%	1.50 - 2.74%	0.62
		Docosahexaenoic acid (DHA, C22:6n3)	3.84%	4.29 - 5.91%	0.81
	Omega-6	**Omega-6 Fatty Acids**	**33.69%**		
		Linoleic (C18:2n6)	21.63%	13.79 - 21.23%	3.72
		Gamma-Linolenic (C18:3n6)	0.09%	0.04 - 0.16%	0.06
		Eicosadienoic (C20:2n6)	0.23%	0.15 - 0.25%	0.05
		Dihomo-γ-linolenic (C20:3n6)	1.11%	0.71 - 1.33%	0.31
		Arachidonic (AA, C20:4n6)	9.46%	7.03 - 9.47%	1.22
		Docosatetraenoic (C22:4n6)	1.04%	0.46 - 0.88%	0.21
		Docosapentaenoic-n6 (C22:5n6)	0.12%	0.10 - 0.22%	0.06
cis-Monounsaturated Fatty Acids	-	**cis-Monounsaturated Fatty Acids**	**22.65%**		
	Omega-7	Palmitoleic (C16:1n7)	0.58%	0.30 - 1.04%	0.37
	Omega-9	Oleic (C18:1n9)	21.48%	14.45 - 18.89%	2.22
		Eicosenoic (C20:1n9)	0.28%	0.04 - 0.34%	0.15
		Nervonic (C24:1n9)	0.31%	0.20 - 0.64%	0.22
Saturated Fatty Acids		**Saturated Fatty Acids**	**34.61%**		
		Myristic (C14:0)	0.39%	0.26 - 0.84%	0.29
		Palmitic (C16:0)	20.85%	22.29 - 26.73%	2.22
		Stearic (C18:0)	12.65%	12.88 - 19.14%	3.13
		Arachidic (C20:0)	0.16%	0.14 - 0.34%	0.1
		Behenic (C22:0)	0.25%	0.21 - 0.47%	0.13
		Lignoceric (C24:0)	0.31%	0.26 - 0.62%	0.18
Trans Fatty Acids		**Trans Fatty Acids**	**0.36%**		
		Trans Palmitoleic (C16:1n7t)	0.14%	0.12 - 0.28%	0.08
		Trans Oleic (C18:1n9t)	0.15%	0.23 - 0.51%	0.14
		Trans Linoleic (C18:2n6t)	0.06%	0.14 - 0.24%	0.05
		Trans Fat Index	0.21%	0.00 - 1.00%	
Ratios		**Ratios**			
		AA:EPA	3.84:1	1.00 - 3.00:1	
		Omega-6:Omega-3	3.88:1	1.00 - 4.00:1	

Model person:
Based on the mean value for every fatty acid, from a group of people fulfilling the following criteria: Omega-3 Index >8%, AA/EPA >3:1, Trans fats <1%. SD value= Standard Deviation. Yellow=>1 SD up or down from model person. Red=>1,5 SD up or down from model person.

COMPLETE FATTY ACIDS REPORT

UK | Europe | Australia
info@fattyacidlabs.com
results.fattyacidlabs.com

Name: Stefan Broselid
DOB: 1985-07-04
Account: Customer

Sample ID: EU9ML6DT
Result date: 2026-02-13

Type		Fatty Acids	Whole Blood Level	Model Person Range	SD Value
Polyunsaturated Fats (PUFAs)	Omega-3	**Omega-3 Fatty Acids**	**8.51%**		
		Omega3 Index	8.47%	8.00 - 12.00%	
		Alpha-Linolenic (C18:3n3)	0.27%	0.08 - 0.68%	0.3
		Eicosapentaenoic (EPA, C20:5n3)	2.47%	2.93 - 4.21%	0.64
		Docosapentaenoic-n3 (C22:5n3)	1.96%	1.50 - 2.74%	0.62
		Docosahexaenoic acid (DHA, C22:6n3)	3.8%	4.29 - 5.91%	0.81
	Omega-6	**Omega-6 Fatty Acids**	**34.48%**		
		Linoleic (C18:2n6)	22.07%	13.79 - 21.23%	3.72
		Gamma-Linolenic (C18:3n6)	0.09%	0.04 - 0.16%	0.06
		Eicosadienoic (C20:2n6)	0.18%	0.15 - 0.25%	0.05
		Dihomo-γ-linolenic (C20:3n6)	0.99%	0.71 - 1.33%	0.31
		Arachidonic (AA, C20:4n6)	10.05%	7.03 - 9.47%	1.22
		Docosatetraenoic (C22:4n6)	0.97%	0.46 - 0.88%	0.21
		Docosapentaenoic-n6 (C22:5n6)	0.12%	0.10 - 0.22%	0.06
cis-Monounsaturated Fatty Acids	-	**cis-Monounsaturated Fatty Acids**	**22.06%**		
	Omega-7	Palmitoleic (C16:1n7)	0.5%	0.30 - 1.04%	0.37
	Omega-9	Oleic (C18:1n9)	20.99%	14.45 - 18.89%	2.22
		Eicosenoic (C20:1n9)	0.21%	0.04 - 0.34%	0.15
		Nervonic (C24:1n9)	0.36%	0.20 - 0.64%	0.22
Saturated Fatty Acids		**Saturated Fatty Acids**	**34.55%**		
		Myristic (C14:0)	0.48%	0.26 - 0.84%	0.29
		Palmitic (C16:0)	20.65%	22.29 - 26.73%	2.22
		Stearic (C18:0)	12.54%	12.88 - 19.14%	3.13
		Arachidic (C20:0)	0.19%	0.14 - 0.34%	0.1
		Behenic (C22:0)	0.34%	0.21 - 0.47%	0.13
		Lignoceric (C24:0)	0.35%	0.26 - 0.62%	0.18
Trans Fatty Acids		**Trans Fatty Acids**	**0.4%**		
		Trans Palmitoleic (C16:1n7t)	0.15%	0.12 - 0.28%	0.08
		Trans Oleic (C18:1n9t)	0.15%	0.23 - 0.51%	0.14
		Trans Linoleic (C18:2n6t)	0.11%	0.14 - 0.24%	0.05
		Trans Fat Index	0.26%	0.00 - 1.00%	
Ratios		**Ratios**			
		AA:EPA	4.07:1	1.00 - 3.00:1	
		Omega-6:Omega-3	4.05:1	1.00 - 4.00:1	

Model person:
Based on the mean value for every fatty acid, from a group of people fulfilling the following criteria: Omega-3 Index >8%, AA/EPA >3:1, Trans fats <1%. SD value= Standard Deviation. Yellow=>1 SD up or down from model person. Red=>1,5 SD up or down from model person.

COMPLETE FATTY ACIDS REPORT

ArcticMed

Name: Stefan Broselid
DOB: 1985-07-04
Account: Customer

Sample ID: EUNSTIRV
Result date: 2026-06-25

UK | Europe | Australia
info@fattyacidlabs.com
results.fattyacidlabs.com

Type		Fatty Acids	Whole Blood Level	Model Person Range	SD Value
Polyunsaturated Fats (PUFAs)	Omega-3	**Omega-3 Fatty Acids**	**9.83%**		
		Omega3 Index	9.84%	8.00 - 12.00%	
		Alpha-Linolenic (C18:3n3)	0.32%	0.08 - 0.68%	0.3
		Eicosapentaenoic (EPA, C20:5n3)	3.44%	2.93 - 4.21%	0.64
		Docosapentaenoic-n3 (C22:5n3)	2.04%	1.50 - 2.74%	0.62
		Docosahexaenoic acid (DHA, C22:6n3)	4.04%	4.29 - 5.91%	0.81
	Omega-6	**Omega-6 Fatty Acids**	**30.08%**		
		Linoleic (C18:2n6)	18.93%	13.79 - 21.23%	3.72
		Gamma-Linolenic (C18:3n6)	0.07%	0.04 - 0.16%	0.06
		Eicosadienoic (C20:2n6)	0.19%	0.15 - 0.25%	0.05
		Dihomo-γ-linolenic (C20:3n6)	0.8%	0.71 - 1.33%	0.31
		Arachidonic (AA, C20:4n6)	8.98%	7.03 - 9.47%	1.22
		Docosatetraenoic (C22:4n6)	0.94%	0.46 - 0.88%	0.21
		Docosapentaenoic-n6 (C22:5n6)	0.17%	0.10 - 0.22%	0.06
cis-Monounsaturated Fatty Acids	-	**cis-Monounsaturated Fatty Acids**	**22.68%**		
	Omega-7	Palmitoleic (C16:1n7)	0.74%	0.30 - 1.04%	0.37
	Omega-9	Oleic (C18:1n9)	20.69%	14.45 - 18.89%	2.22
		Eicosenoic (C20:1n9)	0.22%	0.04 - 0.34%	0.15
		Nervonic (C24:1n9)	1.04%	0.20 - 0.64%	0.22
Saturated Fatty Acids		**Saturated Fatty Acids**	**37.17%**		
		Myristic (C14:0)	0.51%	0.26 - 0.84%	0.29
		Palmitic (C16:0)	22.63%	22.29 - 26.73%	2.22
		Stearic (C18:0)	12.39%	12.88 - 19.14%	3.13
		Arachidic (C20:0)	0.18%	0.14 - 0.34%	0.1
		Behenic (C22:0)	0.59%	0.21 - 0.47%	0.13
		Lignoceric (C24:0)	0.85%	0.26 - 0.62%	0.18
Trans Fatty Acids		**Trans Fatty Acids**	**0.24%**		
		Trans Palmitoleic (C16:1n7t)	0.06%	0.12 - 0.28%	0.08
		Trans Oleic (C18:1n9t)	0.12%	0.23 - 0.51%	0.14
		Trans Linoleic (C18:2n6t)	0.06%	0.14 - 0.24%	0.05
		Trans Fat Index	0.18%	0.00 - 1.00%	
Ratios		**Ratios**			
		AA:EPA	2.61:1	1.00 - 3.00:1	
		Omega-6:Omega-3	3.06:1	1.00 - 4.00:1	

Model person:
Based on the mean value for every fatty acid, from a group of people fulfilling the following criteria: Omega-3 Index >8%, AA/EPA >3:1, Trans fats <1%. SD value= Standard Deviation. Yellow=>1 SD up or down from model person. Red=>1,5 SD up or down from model person.

Appendix 2. Analytical Dataset Construction and Methods

This appendix describes the construction of the analytical datasets and the methods used to generate Figures 1–5. The purpose is to provide a transparent link between the original laboratory reports reproduced in Appendix 1 and the derived analyses presented in the main text.

Four longitudinal whole-blood fatty-acid profiles were included:

• June 2025 (Zinzino fatty-acid analysis)

• November 2025 (FattyAcidLabs, laboratory report issued under the ArcticMed reporting format)

• February 2026 (FattyAcidLabs, laboratory report issued under the ArcticMed reporting format)

• June 2026 (FattyAcidLabs, laboratory report issued under the ArcticMed reporting format)

The original laboratory reports were retained unchanged in Appendix 1. The values used for analysis were transcribed from these reports and organized into a harmonized longitudinal dataset.

A2.1 Analytical dataset construction

The analytical workflow consisted of:

Original laboratory reports

↓

Transcription and harmonization of reported fatty-acid variables

↓

Definition of stable longitudinal fatty-acid panel

↓

Standardization of variables where required

↓

Multivariate analysis and transition analysis

↓

Lipid-class interpretation

No missing June 2025 values were imputed.

A2.2 Stable longitudinal fatty-acid panel

A core longitudinal panel was defined using fatty acids measured at all four time points. This stable panel was used for analyses requiring direct temporal comparability, including:

• principal component analysis (PCA)

• hierarchical clustering

• comparable transition-vector magnitude calculations

The core panel consisted of:

- EPA (20:5 n-3)
- DPA-n3 (22:5 n-3)
- DHA (22:6 n-3)
- alpha-linolenic acid (ALA; 18:3 n-3)
- linoleic acid (LA; 18:2 n-6)
- gamma-linolenic acid (GLA; 18:3 n-6)
- dihomo-gamma-linolenic acid (DGLA; 20:3 n-6)
- arachidonic acid (AA; 20:4 n-6)
- oleic acid (18:1 n-9)
- palmitic acid (16:0)
- stearic acid (18:0)

A2.3 Extended fatty-acid panel

Additional fatty acids available only in later FattyAcidLabs measurements were treated as exploratory extended-panel variables.

Nervonic acid (24:1 n-9) was included in the extended panel because of its biological relevance to membrane lipid biology. However, because no June 2025 measurement was available, nervonic acid was excluded from the primary four-time-point PCA, clustering, and core transition magnitude calculations.

Nervonic acid analyses therefore describe an exploratory three-time-point trajectory from November 2025 onward.

A2.4 Principal component analysis

PCA was performed on the standardized 11-fatty-acid core panel.

Fatty-acid abundances were transformed into z-scores across the longitudinal dataset to place variables with different abundance ranges onto a comparable scale.

PCA was used to visualize relationships between longitudinal fatty-acid composition configurations. Each measurement time point was projected into principal component space based on the combined pattern of measured fatty acids.

The first two principal components explained 61.0% and 35.6% of observed variance, respectively.

The PCA trajectory represents movement within measured fatty-acid composition space. It does not represent biological improvement, deterioration, ECS activity, or physiological adaptation.

A2.5 Hierarchical clustering

Hierarchical clustering was performed using the same standardized 11-fatty-acid core matrix used for PCA.

Euclidean distance and Ward linkage were applied to evaluate relative similarity among longitudinal fatty-acid configurations.

The clustering analysis provides an independent visualization of compositional relationships and does not define validated biological states or categories.

A2.6 Transition-vector analysis

Consecutive longitudinal transitions were quantified using standardized changes in the core fatty-acid panel:

• June 2025 → November 2025

• November 2025 → February 2026

• February 2026 → June 2026

For each interval, the standardized difference for each fatty acid was calculated.

Euclidean vector magnitude was used as a descriptive measure of total movement within the standardized 11-dimensional fatty-acid space.

Directional contributions identify which fatty acids contributed to movement during each interval.

Core vector magnitude calculations were restricted to the stable panel to preserve comparability across all time points.

A2.7 Lipid-class analysis

The February 2026 to June 2026 transition was additionally examined at the lipid-class level.

Selected measured fatty acids were grouped into constructed lipid classes corresponding to the Figure 5 decomposition: omega-3 fatty acids (EPA, DPA-n3, DHA), omega-6 fatty acids (linoleic acid [LA], dihomo-gamma-linolenic acid [DGLA], arachidonic acid [AA]), monounsaturated fatty acids (MUFA; oleic acid and nervonic acid where available), and saturated fatty acids (SFA; palmitic acid and stearic acid). This analysis provides a higher-level representation of compositional redistribution across the measured fatty-acid panel.

Because fatty-acid measurements are compositional, changes in one class necessarily alter the relative contribution of other classes. Class-level analysis therefore describes redistribution within the measured composition and should not be interpreted as independent biological effects or absolute changes in lipid mass.

A2.8 Interpretation boundaries

The analytical procedures described here are exploratory and descriptive.

They are intended to determine whether repeated fatty-acid profiles can reveal structured longitudinal compositional movement.

They do not establish:

• causal relationships

• validated lipid-state categories

• ECS activation

• membrane composition in specific tissues

• receptor function

• physiological benefit

The analytical framework provides a transparent method for representing longitudinal lipid composition and generating hypotheses for future controlled studies.

Table A2.1. Core longitudinal fatty-acid dataset

FATTY ACID	JUNE 2025	NOVEMBER 2025	FEBRUARY 2026	JUNE 2026
EPA (20:5 N-3)	1.97	2.47	2.47	3.44
DPA-N3 (22:5 N-3)	1.66	2.12	1.96	2.04
DHA (22:6 N-3)	3.00	3.84	3.80	4.04
ALA (18:3 N-3)	0.64	0.27	0.27	0.32
LINOLEIC ACID, LA (18:2 N-6)	22.00	21.63	22.07	18.93
GLA (18:3 N-6)	0.08	0.09	0.09	0.07
DGLA (20:3 N-6)	1.09	1.11	0.99	0.80
ARACHIDONIC ACID, AA (20:4 N-6)	8.40	9.46	10.05	8.98
OLEIC ACID (18:1 N-9)	24.40	21.48	20.99	20.69
PALMITIC ACID (16:0)	22.90	20.85	20.65	22.63
STEARIC ACID (18:0)	13.80	12.65	12.54	12.39

Table A2.2. Extended nervonic-acid trajectory

Fatty acid	June 2025	November 2025	February 2026	June 2026
Nervonic acid (24:1 n-9)	Not available	0.31	0.36	1.04

Table A2.3. PCA coordinates of the four longitudinal configurations

TIME POINT	PC1	PC2
JUNE 2025	4.404	-0.642
NOVEMBER 2025	-0.926	1.736

FEBRUARY 2026	-1.215	1.866
JUNE 2026	-2.263	-2.960

Table A2.4. Core longitudinal transition magnitudes

TRANSITION	STANDARDIZED VECTOR MAGNITUDE
JUNE 2025 → NOVEMBER 2025	5.91
NOVEMBER 2025 → FEBRUARY 2026	1.75
FEBRUARY 2026 → JUNE 2026	5.01

Table A2.5. Major fatty-acid class totals

Lipid class	**February 2026**	**June 2026**	**Absolute change**
Omega-3 total	8.23	9.52	+1.29 percentage points
Omega-6 total	33.11	28.71	-4.40 percentage points
MUFA total	21.35	21.73	+0.38 percentage points
SFA total	33.19	35.02	+1.83 percentage points

Values represent the summed contribution of constituent fatty acids included in each constructed class and therefore differ from laboratory-reported lipid-class summaries.