
Molecular Biomarkers of Training Maladaptation: A Multi-Omics Roadmap from Functional Overreaching to Overtraining Syndrome

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Review

Molecular Biomarkers of Training Maladaptation: A Multi-Omics Roadmap from Functional Overreaching to Overtraining Syndrome

Dan Cristian Mănescu ^{1,*}, Andreea Voinea ^{1,*}, Camelia Daniela Plastoi ², Alexandra Reta Iacobini ³, Alina Anca Vulpe ⁴, Ancuța Pîrvan ⁵, Corina Claudia Dinciu ¹, Bogdan Iulian Vulpe ⁴, Cristian Băltărețu ^{6,*} and Adrian Iacobini ¹

¹ Department of Physical Education and Sports, Bucharest University of Economic Studies, 010374 Bucharest, Romania

² Sport and Health Department, Faculty of Medical and Behavioral Sciences, Constantin Brâncuși University of Târgu-Jiu, 210135 Târgu-Jiu, Romania

³ Department of Physical Education and Sports, Faculty of Physical Education and Sport, Spiru Haret University, 030045 Bucharest, Romania

⁴ Department of Physical Education and Sports, Technical University of Civil Engineering Bucharest, 020396 Bucharest, Romania

⁵ Department of Physical Education and Sports, Faculty of Humanities, Valahia University of Târgoviște, 130105 Târgoviște, Romania

⁶ Doctoral School, National University of Physical Education and Sport, 060057 Bucharest, Romania; Romanian Bodybuilding and Fitness Federation (FRCF), 030167 Bucharest, Romania

* Correspondence: dan.manescu@defs.ase.ro (D.C.M); andreea.voinea@defs.ase.ro (A.V.); baltaretuc@gmail.com (C.B.)

Abstract

Exercise adaptation depends on overload that is resolved by recovery, yet the same biology becomes maladaptive when immune, endocrine, metabolic, and muscle-centered stress signals fail to normalize. Exercise-induced maladaptation represents a systems-level failure of biological resolution, with direct relevance to disease-like dysregulation. Functional overreaching, non-functional overreaching, and overtraining syndrome remain difficult to diagnose because no single biomarker provides adequate specificity, temporal stability, or clinical portability. This narrative review synthesizes human and mechanistic evidence across proteomics, transcriptomics, metabolomics, endocrine profiling, extracellular vesicles, and mitochondrial quality-control biology to define the molecular architecture most relevant to athlete monitoring. Across these layers, the most coherent signatures cluster in immune-acute-phase activation, redox-buffering strain, endocrine drift, altered substrate availability, excitation-contraction dysfunction, integrated stress-response signaling, and defects in autophagy-mitophagy and lysosomal remodeling. Three translational elements emerge from this synthesis: a systems-convergence model of recovery failure, a staged biomarker deployment hierarchy, and a provisional Recovery Failure Index. The practical priority is therefore not a solitary marker, but serial phenotype-anchored multimarker panels that connect circulating signals with muscle-centered biology and support decision-making before prolonged recovery failure becomes entrenched.

Keywords: overtraining syndrome; functional overreaching; non-functional overreaching; molecular biomarkers; multi-omics; athlete monitoring; proteomics; metabolomics; mitochondrial stress; recovery failure index

1. Introduction

Exercise adaptation is a controlled biological wager: overload is imposed to disturb homeostasis, yet the intended reward-improved function appears only if repair, substrate repletion, and neuroendocrine coordination are allowed to catch up. In the applied setting, the same training stimulus may be productive in one athlete and harmful in another because nutrition, sleep, illness burden, psychosocial stress, travel, environmental load, and competition density alter the organism's capacity to resolve perturbation [1,2]. The central coaching and clinical problem is therefore not whether overload should occur, but when biological strain remains adaptive and when it begins to produce recovery failure.

The continuum linking adaptive overload, functional overreaching (FOR), non-functional overreaching (NFOR), and overtraining syndrome (OTS) is conceptually familiar but operationally difficult. FOR is usually viewed as a deliberately induced, short-lived decrement that resolves after rest and may be followed by supercompensation. NFOR reflects a more persistent decrement with delayed recovery, whereas OTS denotes a prolonged state of underperformance accompanied by fatigue and multisystem disturbance [1,2]. These states are not separated by a single threshold molecule or a single symptom; rather, they emerge from interacting immune, endocrine, metabolic, neural, and muscle-centered processes that unfold across different time scales.

That gap between concept and diagnosis remains the field's most persistent weakness. Recent reviews and cohort studies continue to emphasize that OTS has no gold-standard diagnostic test and that current practice still relies on performance decline, symptom history, and exclusion of competing medical explanations [3–6]. At the same time, the molecular literature has become substantially richer. Proteomics has revealed reproducible immune-related signatures during functional overreaching [7,8]; endocrine studies have highlighted shifts in testosterone, estradiol, catecholamines, lactate, and creatine kinase [9,10]; and mechanistic work has connected excessive loading with altered muscle contractile function, calcium sensitivity, mitochondrial stress signaling, and quality-control failure [11–14].

This review is built around a translational question that sits squarely at the intersection of molecular exercise biology and sport monitoring: which molecular signatures are most informative for identifying unresolved training stress before the athlete becomes trapped in a long recovery cycle, and how should those signals be staged in practice [4–6,15–17]? To answer that question, we move from phenotype definition and operational boundaries to immune, endocrine, metabolic, muscle, and mitochondrial domains, and then toward a practitioner-oriented framework in which biomarker interpretation is serial, multimarker, and context-aware rather than reductionist [18–21]. We also introduce a provisional Recovery Failure Index (RFI) and a deployment hierarchy intended to separate field-ready monitoring from escalation assays and research-stage liquid biopsies.

The review is guided by five explicit questions stated below:

Q1. Which molecular programs distinguish productive overload from unresolved maladaptation, and at what point does normal adaptation cease to be biologically efficient?

Q2. Which biospecimens and molecular layers are most informative in the early transition from functional overreaching to prolonged recovery failure: routine blood chemistry, endocrine panels, proteomics, metabolomics, or secreted vesicle cargo?

Q3. Why have single biomarkers repeatedly failed to generate stable diagnostic thresholds, and why does the biology of training maladaptation favour multimarker interpretation anchored to phenotype and sampling time?

Q4. How do mitochondrial stress, redox imbalance, proteostasis, lysosomal signaling, autophagy-mitophagy, excitation-contraction coupling, and inter-organ communication converge to create the muscle-centered phenotype of maladaptation?

Q5. How should biomarker studies be designed so that molecular signatures become usable for athlete monitoring rather than remain mechanistic observations without practical interpretability?

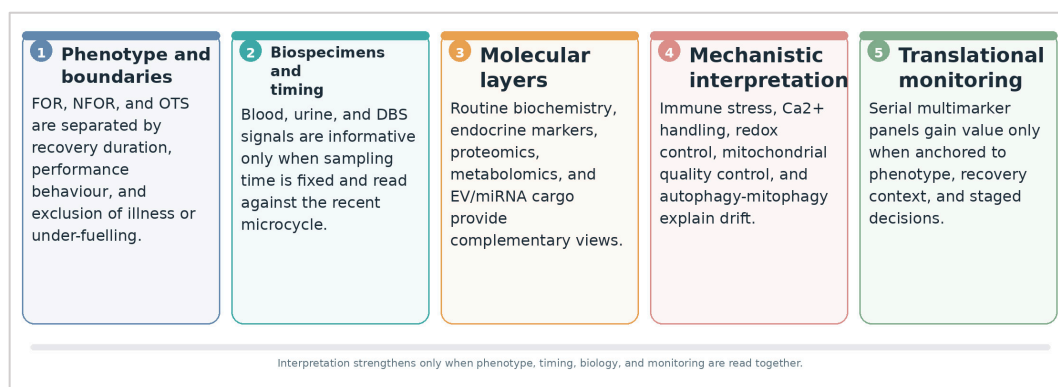


Figure 1. Architecture of the review. The manuscript is organized across five linked levels: phenotype and operational boundaries, biospecimens and timing, molecular layers, mechanistic interpretation, and translational decisions. The layered sequence reflects the diagnostic-to-monitoring progression emphasized in current overtraining and athlete-biomarker syntheses [4,6,16].

Here we argue that training maladaptation is best understood as a systems-level failure of recovery biology rather than as an extreme of training load alone, a framing that is also compatible with recent conceptual work on oscillatory load, energetic strain, and molecular plasticity [5,17,22]. In this context, excessive exercise stress emerges as a biologically informative model of unresolved molecular and physiological dysregulation. This review introduces three integrative contributions intended to bridge molecular biology and applied athlete monitoring: (1) a systems-convergence model of recovery failure linking multi-domain stress signals to failed biological closure; (2) a staged biomarker deployment hierarchy distinguishing field-ready markers from escalation and research-stage assays; and (3) a provisional Recovery Failure Index (RFI) designed to support serial, phenotype-anchored decision-making. Together, these elements aim to move the field beyond descriptive biomarker lists toward an operational framework that is both mechanistically grounded and practically interpretable.

1.1. Literature Review Strategy and Scope

This narrative review was assembled through structured PubMed searches updated to March 2026 using combinations of the terms 'functional overreaching', 'non-functional overreaching', 'overtraining syndrome', 'exercise proteomics', 'exercise metabolomics', 'skeletal muscle transcriptomics', 'mitochondrial stress response', 'autophagy', 'mitophagy', 'oxidative stress', 'reactive oxygen species', 'redox signaling', 'Nrf2/Keap1', 'extracellular vesicles', 'microRNA', 'RED-S', and 'low energy availability'. Backward citation tracking was used to capture seminal definitions, mechanistic anchors, and contemporary multi-omics studies. The search was designed to prioritize mechanistic relevance and translational interpretability rather than exhaustive study counting.

Priority was given to peer-reviewed human studies in trained or competitive populations, longitudinal or repeated-sampling designs, muscle-biopsy or omics datasets, and reports explicitly linked to performance, fatigue, delayed recovery, or clinical phenotype. Reviews, consensus statements, and foundational physiological papers were retained when they clarified definitions, pathway logic, or methodological constraints. Animal and cell studies were used selectively for mechanistic interpretation where direct human evidence remains limited. The literature was organized into five thematic blocks: (1) conceptual and diagnostic frameworks for FOR, NFOR, and OTS; (2) exercise transcriptomics, proteomics, metabolomics, and sportomics resources; (3) mitochondrial quality control, calcium handling, autophagy, lysosomal signaling, and integrated stress-response biology; (4) extracellular vesicles, exerkines, and circulating microRNAs; and (5) the endocrine-metabolic interface represented by low energy availability and RED-S-related biomarker studies. This was designed as a structured narrative synthesis rather than a formal systematic review, with an emphasis on mechanistic integration and translational relevance. No PRISMA-based

screening workflow was applied because the aim was integration and framework building rather than quantitative evidence aggregation.

The evidence base is also asymmetric in depth. Consensus and diagnostic papers provide the operational vocabulary for FOR, NFOR, and OTS, whereas the strongest molecular detail often comes from small overload models, case studies, or mechanistic muscle studies. Large transcriptomic, proteomic, and consortium-scale resources now offer a broader reference frame for what coordinated exercise-responsive biology looks like in humans, but clinically well-phenotyped OTS cohorts remain rare. The synthesis below therefore gives greatest weight to phenotype-anchored human data and uses preclinical work primarily to interpret pathway directionality rather than to infer clinical thresholds.

Accordingly, the review was structured not only to identify candidate biomarkers of maladaptation, but also to clarify how exercise-induced recovery failure can be interpreted within broader mechanisms of stress integration and biological resolution.

2. From Adaptive Overload to Training Maladaptation

2.1. Definitions and Operational Boundaries

A useful starting point is to abandon the idea that FOR, NFOR, and OTS are isolated boxes. They are better understood as states distributed along a continuum of biological strain and incomplete recovery. What separates them is not simply symptom severity, but the duration of impaired performance, the speed of recovery after load reduction, and the extent to which signals spread from a local training response to a multisystem pattern involving immune, endocrine, metabolic, autonomic, and psychological domains [1,2,5].

The most practical distinction is temporal and functional. In FOR, the athlete tolerates a brief decrement because the subsequent recovery period is built into the plan. In NFOR, recovery takes longer than expected and the athlete begins to lose confidence in the normal relationship between load and rebound. In OTS, the decrement becomes persistent, symptoms broaden, and the athlete no longer behaves like a temporarily fatigued performer but like an organism that has lost normal conditioning responses [9,10]. This loss-of-conditioning concept is important because it moves the discussion away from the simplistic notion that OTS is merely 'too much training' and toward the more accurate view that maladaptation emerges from a mismatch between external load and total recovery environment. This progression is summarized schematically in Figure 2, which positions adaptive overload, FOR, NFOR, and OTS along a continuum of decreasing recovery reserve and increasing load-recovery mismatch [1,4–6].

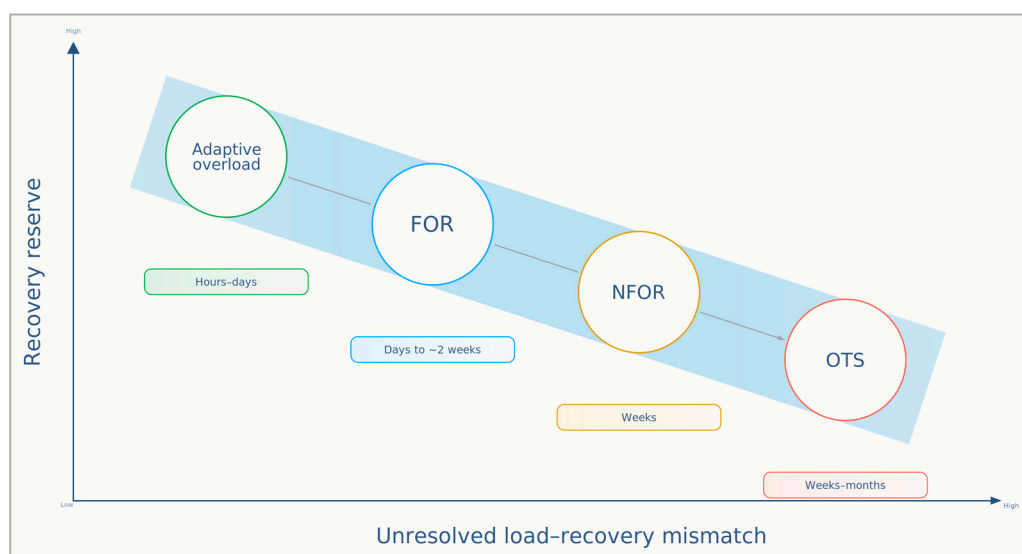


Figure 2. Conceptual schematic continuum from adaptive overload to overtraining syndrome (OTS). States are positioned along a descending band of recovery reserve as unresolved load-recovery mismatch increases from

left to right. Circles denote adaptive overload, functional overreaching (FOR), non-functional overreaching (NFOR), and OTS; the labels below indicate pragmatic recovery windows (hours-days, days to ~2 weeks, weeks, and weeks-months). The vertical and horizontal axes are qualitative rather than quantitative and are intended to indicate directional change only. The schematic emphasizes shrinking rebound capacity and progressively greater biological uncoupling across the continuum [1,2,4–6].

Table 1 should therefore be read as an operational continuum rather than as a rigid diagnostic ladder. Its main value is to distinguish a planned, reversible perturbation from a state in which recovery time itself becomes biologically informative [1,4–6].

Table 1. Operational continuum linking productive overload to overt training maladaptation.

Practical interpretation	Dominant biological picture	Recovery window	Performance profile	State
Expected training response	Transient stress signaling matched by recovery	Hours to a few days	Stable or improving	Adaptive overload
Can be planned	Reversible immune-proteomic perturbation with retained adaptive capacity	Days to about 2 weeks	Short-term decrement	Functional overreaching
Requires deload and follow-up	Multidomain disturbance and delayed normalization	Weeks	Persistent decrement	Non-functional overreaching
Clinical work-up and staged rebuild	Endocrine, immune, metabolic, and clinical loss of conditioning	Many weeks to months	Long-lasting underperformance	Overtraining syndrome

Note: FOR, functional overreaching; NFOR, non-functional overreaching; OTS, overtraining syndrome. The states shown are pragmatic categories along a continuum rather than discrete biological entities, and recovery windows are approximate and influenced by sport discipline, competition density, energy availability, sleep, illness burden, and other recovery conditions [1,2,4–6].

2.2. Limitations of Current Diagnostic Approaches

Three methodological problems keep the field stuck. First, studies rarely capture the full phenotype. A biomarker cannot be interpreted well if the accompanying information on recent load, illness, caloric intake, sleep, menstrual or hormonal status, travel, and psychological stress is poor. Second, many studies examine a single time point even though maladaptation is inherently longitudinal. Third, the biological signal is often anchored to a broad label such as 'fatigue' without rigorous performance phenotyping [3–6].

These limitations help explain why the literature contains both exciting findings and persistent uncertainty. A marker may be biologically meaningful without being diagnostically decisive. This review therefore treats candidate biomarkers not as standalone tests, but as contributors to a serial interpretation model in which biological data gain meaning only when integrated with the athlete's recent trajectory [4,6,16].

3. Immune and Inflammatory Signatures of Maladaptation

3.1. Acute Phase and Innate Immune Signals

Among the most compelling biomarker studies in this space is the proteomic work on functional overreaching by Nieman and colleagues. Using dried blood spot proteomics during a 3-day overload model, the authors identified a 13-protein cluster associated with FOR, most of it linked to the acute phase response and innate immune activation [7]. This study is important not because it solved diagnosis, but because it demonstrated that productive overload leaves a coherent molecular footprint that extends beyond conventional markers such as creatine kinase.

The RAAM case study by Merritt and colleagues reinforced that message under an ultraendurance scenario more consistent with NFOR-like stress. Their targeted proteomic panel highlighted proteins involved in complement activation and the acute phase response, again pointing to immune-related biology rather than a simple muscle-damage narrative [8]. Together, these papers suggest that the blood proteome may detect maladaptive strain earlier and more holistically than traditional single-analyte monitoring.

3.2. Dysregulated Inflammatory Responses in Maladaptation

Inflammation is not inherently pathological in sport; it is part of normal remodeling. The problem begins when the inflammatory signal is prolonged, poorly resolved, or amplified by non-training stressors. In that situation, the same biological systems that support adaptation may instead contribute to sustained fatigue, altered mood, impaired immune competence, and slower tissue recovery [2,5,15].

This is one reason isolated cytokine measurements have often disappointed: the signal is timing-sensitive and strongly shaped by the training session that preceded sampling [16,23,24]. Acute phase proteins, neutrophil-derived proteins, complement-related markers, and simple hematological ratios may therefore be more informative when sampled repeatedly and interpreted as a pattern rather than as independent winners or losers [7,8,16]. The practical implication is straightforward: inflammatory biology remains central, but it has to be monitored longitudinally and in context [4,16].

3.3. Converging Immune-Proteomic Patterns Across Overload Models

Across the human overload literature, convergence is strongest at the level of immune-proteomic patterning rather than single cytokines. Short overload blocks, ultraendurance case studies, and clinically phenotyped OTS cohorts all point toward a recurring cluster composed of acute-phase proteins, complement-related biology, neutrophil-associated signals, and altered simple hematology, even though the exact members of the panel differ by platform and recovery window [4,7,8,10,16,23]. What appears to generalize is not a universal molecule but a biological direction: when overload ceases to be efficiently resolved, recovery-day blood no longer resembles a transient post-exercise perturbation and instead retains features of innate immune activation. This makes immune-proteomic signatures most useful as trajectory markers that distinguish expected rebound from prolonged recovery failure.

3.4. Sources of Divergence and False-Positive Inflammation

At the same time, the inflammatory literature is heterogeneous for good reasons. Cytokines show narrow kinetic windows, and DBS proteomics and venous plasma do not sample identical biology [7,16,23,24]. The same athlete may also present a different inflammatory profile depending on illness exposure, glycogen status, caloric sufficiency, menstrual or hormonal context, travel stress, and the preceding microcycle [1,4,16,17,25,26]. These factors explain why apparently contradictory studies can still be biologically compatible. For translational purposes, the main lesson is methodological: the field should stop asking whether 'inflammation' is present and instead ask

whether immune-related signals remain inappropriately elevated for the athlete's recovery state, phenotype, and sampling window.

Read longitudinally, the domains in Table 2 suggest a tiered monitoring logic: inflammatory and endocrine-metabolic layers are first-line candidates, whereas deeper omics signatures become most useful when routine markers and phenotype remain discordant [3,7,8,10,16]. These layers are hierarchical rather than additive; most athletes will not require simultaneous interrogation of every domain, and escalation should follow persistent phenotype-biology mismatch rather than analytic curiosity alone, ideally within transparent decision frameworks that remain physiologically interpretable [16,24,27].

Table 2. Candidate biomarker domains most relevant to the detection of training maladaptation.

Evidence base	Interpretation caveat	Typical pattern in maladaptation	Candidate signals	Domain
Moderate human	Strongly timing-dependent; recent illness can mimic the pattern	Signal persistence or exaggerated recovery-day elevation	Acute phase proteins, complement-related proteins, neutrophil-derived proteins, leukocyte ratios	Immune / inflammatory
Moderate human	Basal values may appear 'normal' relative to reference ranges	Loss of athlete-like conditioning profile	Testosterone, estradiol, testosterone:estradiol ratio, catecholamines	Endocrine
Moderate human	Session design and training type influence direction and magnitude	Disproportionate metabolic or contractile strain	Lactate, creatine kinase, submaximal force measures, Ca ²⁺ sensitivity-related surrogates	Metabolic / muscle
Emerging human	Platform harmonization is still limited	Composite shifts outperform single molecules	Targeted protein panels, metabolite panels, integrated signatures	Proteomics / multi-omics
Strong applied	Biomarkers are weak if not tied to function	Necessary for phenotype anchoring	Performance tests, symptom scores, perceived effort, recovery duration	Clinical anchor

Note: The domains are presented as serial monitoring layers rather than standalone diagnostic tests. Evidence-base labels reflect translational maturity, not universal diagnostic thresholds. Ca²⁺, calcium. All signals should be interpreted against recent load, illness exposure, energy availability, and the athlete's symptom and performance trajectory [7,8,10,16,24].

4. Endocrine, Metabolic, and Muscle-Centered Stress Signatures

4.1. Hormonal and Biochemical Patterns

The EROS series remains one of the most informative human programs for understanding the multisystem signature of OTS. In the EROS-BASAL study, OTS-affected male athletes showed lower testosterone and neutrophils, higher estradiol, catecholamines, lactate, and creatine kinase, and a markedly reduced testosterone:estradiol ratio compared with healthy athletes [10]. The key lesson is not that one hormone becomes diagnostic, but that healthy athletic conditioning itself carries a distinct biochemical pattern that can be lost or inverted during maladaptation.

The broader EROS analysis pushed this idea further by framing OTS as a state of 'paradoxical deconditioning', in which many athlete-favorable adaptations disappear and the biological profile drifts toward that of nonathletic controls [9]. Complementary endocrine work and related reviews have implicated altered HPA-axis behavior, cortisol awakening responses, abnormal non-exercise stress responses, and contextual disruptors as part of that multisystem picture [28–32]. The EROS-DIAGNOSIS paper then showed why composite tools are attractive: when clinical features were combined with basal hormones and, in some versions, stimulation-test responses, discrimination improved markedly within that cohort [3]. Even if those tools still require broader validation, they underscore a principle that likely applies across all biomarker research in sport: single markers are fragile; structured combinations are more robust.

That endocrine picture also has a fuel-sensing counterpart. Bellinger argued that performance-defined functional overreaching is metabolically heterogeneous and may reflect context-dependent combinations of glycogen stress, low energy availability, and incomplete autonomic recovery rather than a single biochemical state [17]. Recent conceptual work on training-fuel coupling extends that logic by arguing that under-fueling and misaligned nutrient timing can convert an otherwise productive training signal into prolonged recovery failure [33]. In keeping with that idea, Coates and colleagues found that overreached endurance athletes displayed lower continuous-glucose-monitoring-derived glucose and suppressed carbohydrate oxidation during standardized submaximal exercise, suggesting that substrate selection and metabolic flexibility may deteriorate before classical damage markers become dramatic [34,35]. For a molecular review, the implication is important: biomarkers of maladaptation should not be limited to damage molecules, but should also report how the muscle partitions and uses fuel under load.

Low energy availability deserves explicit attention because it can biologically magnify many features that are otherwise attributed to training load alone [17,25,26,36–47]. RED-S-related studies consistently show that changes in endocrine tone, glycogen restoration, iron handling, hematological variables, and resting metabolic support can alter the background against which recovery biomarkers are interpreted [25,26,36,41,43,44]. In practice, an athlete may therefore present with immune or hormonal signatures suggestive of training maladaptation while the dominant upstream driver is chronic under-fueling [25,26,41,43,44]. This is one reason why endocrine panels, ferritin or iron-related markers, CK, and inflammatory readouts should never be interpreted without nutritional context [16,25,26,41,43]. Selective supplementation may support remodeling, but it cannot substitute for restoration of energy availability and appropriate training-fuel matching [33,48]. That endocrine-metabolic perspective provides the bridge to the next layer of the review, because unresolved substrate stress is one route by which circulating abnormalities become persistent muscle-centered dysfunction.

4.2. Muscle and Mitochondrial Pathways as Frontier Biomarker Biology

A second frontier lies deeper in the muscle itself, where contractile, metabolic, redox, and mitochondrial signals begin to converge. Classical muscle-fatigue physiology provides the foundation for this view, because prolonged weakness can emerge from interacting changes in excitation-contraction coupling, metabolite handling, and force production [49–51]. Cheng and colleagues emphasized that prolonged low-frequency force depression, altered excitation-contraction

coupling, and impaired mitochondrial support for force production may help explain the stubborn sensation of weakness that accompanies chronic recovery failure [11]. More recent human work on intensified training with insufficient recovery linked impaired neuromuscular function to reduced myofibrillar Ca²⁺ sensitivity, providing a mechanistic bridge between 'the athlete feels flat' and a measurable cellular defect [12].

Preclinical work is now sharpening that bridge. Watanabe and Wada demonstrated that excessive HIIT-like loading can induce myofibrillar force depression in overreaching, while Sanfrancesco and Hood showed that acute contractile activity is sufficient to activate the mitochondrial integrated stress response and ATF4 signaling [13,14]. These mechanisms are not ready-made field biomarkers, but they are highly relevant because they tell us where the next generation of translational signals may emerge: not only in plasma chemistry, but also in markers that report unresolved contractile and mitochondrial stress.

4.3. Redox Biology as the Coupling Layer Between Overload and Recovery

Redox biology sits at the interface between useful adaptation and maladaptive spillover. During exercise, reactive oxygen species are not merely by-products of damage; they act as compartmentalized second messengers that interact with AMPK, p38 MAPK, NF- κ B-linked transcription, and programs governing antioxidant defense and mitochondrial remodeling [52–57]. A practical way to interpret this layer is through an AMPK-mTOR-SIRT1 regulatory triad: AMPK and SIRT1 coordinate fuel stress and mitochondrial remodeling, whereas mTOR helps determine whether the post-exercise state proceeds toward repair and anabolic closure or remains constrained by unresolved energetic strain [53,56,58–64]. A related systems model proposes that repeated oscillation across AMPK-mTOR signaling and NAD⁺ economy may help explain how adaptive signaling drifts toward metabolic overdrive when recovery is chronically incomplete [65]. NOX-derived cytosolic ROS is required for normal GLUT4 translocation and exercise-stimulated glucose uptake, underscoring that redox signaling belongs to adaptation rather than to generic oxidative noise [53]. In human skeletal muscle, exercise to exhaustion rapidly activates the Nrf2/Keap1 axis together with AMPK- and p62-linked regulation, while animal work indicates that p62/SQSTM1 and Nrf2 cooperate to increase antioxidant protein expression in oxidative muscle [54,55].

The translational issue is therefore not whether oxidants rise, but whether redox control re-closes on schedule. Failed recovery is more likely to appear as recovery-day persistence of oxidative pressure, insufficient Nrf2-linked buffering, continued cross-talk with inflammatory and integrated stress-response pathways, and loss of metabolic efficiency, rather than as one abnormal oxidation product [54–57]. Human data also suggest that higher aerobic fitness is associated with a stronger basal antioxidant milieu in skeletal muscle, so the same absolute oxidative burden can carry different biological meanings across athletes [56]. Redox biology is therefore best interpreted as a context-setting layer that modulates immune, endocrine, and mitochondrial readouts instead of as a stand-alone oxidative-stress assay [57].

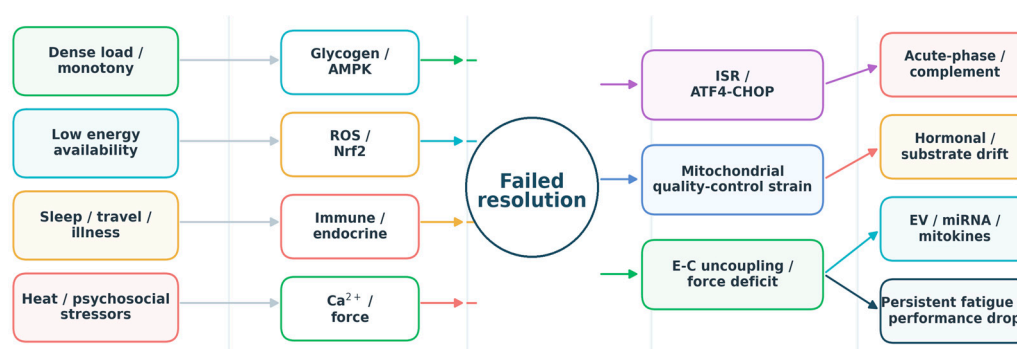


Figure 3. Systems-convergence model of training maladaptation. Contextual stressors feed signal domains involving energy sensing, redox control, immune-endocrine regulation, and contractile/Ca²⁺ handling, which

converge on a failed-resolution hub. Persistent activation is then expressed through ISR/ATF4-CHOP signaling, mitochondrial quality-control strain, and excitation-contraction uncoupling, with downstream readouts in blood, secreted factors, and performance phenotype [11–14,53–55,66]. Illustrative signals mapping onto these nodes include glucose/CGM and lactate (energy sensing), acute-phase or complement-related proteins and leukocyte ratios (immune-endocrine spillover), CK or submaximal force behavior (contractile strain), and exploratory GDF15/FGF21 or EV-linked signals (mitochondrial spillover) [7,8,10,16,35,67]. Arrows indicate dominant left-to-right routes rather than exclusive one-to-one mappings. Abbreviations: Ca²⁺, calcium; ISR, integrated stress response; ATF4, activating transcription factor 4; CHOP, C/EBP homologous protein.

4.4. Proteostasis, Lysosomal Remodeling, and Mitophagy

The next molecular layer concerns quality control rather than force output alone. Repeated contractile stress with incomplete substrate repletion can sustain Ca²⁺, AMPK, and redox pressure, promote eIF2alpha phosphorylation, and activate ATF4/CHOP-linked ISR signaling that interfaces with lysosomal remodeling, autophagy-mitophagy flux, and mitochondrial protein-quality control [14,66,68–73]. In adaptive training this sequence is brief and restorative; in maladaptation it remains open long enough that an initially protective program becomes evidence of unresolved organelle stress.

The key distinction is duration and coupling. These pathways are not pathological because they turn on; they become maladaptive when remodeling, clearance, and rebuilding fail to re-synchronize. The resulting phenotype is plausible: excessive proteolysis, inefficient organelle clearance, declining contractile efficiency, and persistent spillover of stress signals into circulation [11,13,66]. This is why markers linked to eIF2alpha-ATF4 signaling, lysosome-autophagy regulation, FOXO-linked protein breakdown, or mitophagy mediators such as BNIP3, BNIP3L/NIX, and PINK1/Parkin remain mechanistically important even before they become field-ready biomarkers.

At finer resolution, mitochondrial distress is shaped by network remodeling, protein import, and inter-organelle contact biology. Exercise responses increasingly implicate mitochondrial dynamics, import-related UPRmt signaling, ER-mitochondria contact sites, and Mfn2-dependent quality-control programs [70–72,74–86]. A chronically overloaded fibre does not simply accumulate damage; it can lose the coordination required to segregate dysfunctional mitochondrial domains, maintain proteostasis, and restore excitation-contraction homeostasis after repeated loading bouts [11–14].

Mitochondrial stress is also not confined to the fibre. Metabolites, vesicles, GDF15, FGF21, and cell-free mitochondrial DNA may carry unresolved organelle stress into circulation and help connect intramuscular dysfunction with low-grade inflammatory and fatigue phenotypes [67]. These signals remain exploratory in sport, but they offer a biologically coherent bridge between tissue-level quality-control failure and blood-based monitoring. The critical distinction is whether these loops close after recovery or remain biologically open, with persistent spillover into circulation.

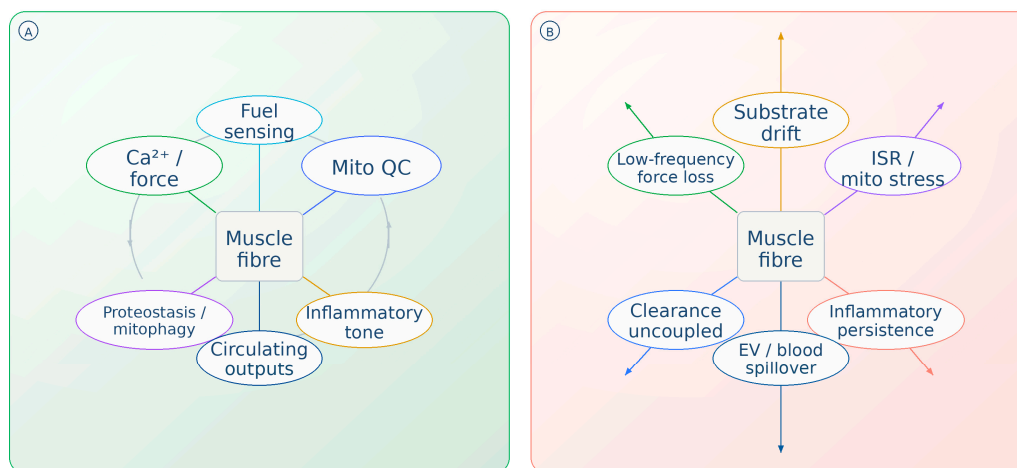


Figure 4. Closed-loop versus open-loop remodeling in skeletal muscle. (A) Resolved adaptation, where fuel sensing, force handling, mitochondrial quality control, proteostasis-mitophagy, inflammatory tone, and

circulating outputs re-equilibrate around the muscle fibre after recovery. (B) Persistent maladaptation, where the same network remains engaged but opens outward into substrate drift, ISR/mitochondrial stress, inflammatory persistence, impaired clearance, force loss, and extracellular/blood spillover [11–14,66–69,73]. Representative outputs of loop closure include normalization of symptoms, performance, and first-line blood markers, whereas open-loop persistence may retain CK/lactate drift, acute-phase or complement signals, altered glucose handling, and exploratory mitochondrial-stress or EV readouts [7,8,10,16,35,67]. Identical geometry across panels emphasizes altered coupling and failure of loop closure rather than a wholly different pathway set. Abbreviations: ISR, integrated stress response.

4.5. Extracellular Vesicles, microRNAs, and the Exercise Secretome

EVs and circulating microRNAs are attractive because they may report tissue stress before routine resting markers diverge [87–90], but they are also among the most pre-analytically fragile layers in exercise biology [91–94]. Exercise modality, sampling window, isolation strategy, storage, matrix choice, and hemolysis can all reshape the signal [90,93]. For maladaptation research, that means EV or miRNA data are best treated as biologically rich second-line layers rather than as frontline diagnostic tests [24,90,93,95].

Their biological appeal is strong. Acute heavy resistance exercise altered 34 EV microRNAs with predicted targets in IGF-I, STAT3, PPAR, JAK/STAT, ERK/MAPK, AMPK, mTOR, and PI3K/AKT pathways, placing EV cargo at the intersection of anabolic signaling, metabolism, and immune regulation [96]. Exercise to exhaustion likewise increased urinary EV abundance and altered EV-microRNA cargo with links to PI3K-Akt, MAPK, and insulin signaling [95].

These findings support a simple translational rule: EV and microRNA layers are most useful when first-line monitoring remains abnormal or biologically discordant after adequate deload, and only when pre-analytical control is strict. Used in that position, they may function as liquid biopsies of unresolved tissue stress rather than as universal screening tools.

The importance of Table 3 is mechanistic rather than diagnostic. Its added timing and feasibility cues are intended to show when a signal is most likely to emerge and how realistically it can be captured in the field. As the signal moves from fuel sensing toward organelle quality-control failure, accessible blood readouts still need to be interpreted alongside performance phenotyping and, in research settings, tissue-level assays [11–14,21,24,66,97].

Table 3. Muscle-centered molecular pathways relevant to training maladaptation, their translational readouts, earliest detection window, and field feasibility.

Domain	Key readouts	Meaning in maladaptation	Biospecimen / maturity	First signal	Field feasibility
Fuel sensing / energetic stress	glucose, lactate, AMPK-linked signatures	reduced metabolic flexibility	blood or CGM; moderate	session-24 h	High
Ca²⁺ handling / contractile apparatus	Ca ²⁺ sensitivity, force-frequency shift	cellular basis of the 'flat' phenotype	advanced physiology or biopsy; low	24-72 h	Low-med.
Redox-inflammatory persistence	acute phase proteins, complement, MPO, ROS/RNS-related tone	failure to resolve innate immune activation	plasma, serum, DBS; moderate	recovery day-72 h	Medium
Mitochondrial ISR / quality control	eIF2 α -ATF4, CHOP, ATF5, GDF15, FGF21	mitochondrial stress with loss of resilient remodeling	biopsy plus exploratory blood markers; low	24 h-block accumulation	Low
Autophagy-lysosome / mitophagy	TFEB/TFE3, BNIP3, BNIP3L/NIX, PINK1, Parkin	organelle turnover uncoupled from effective recovery	muscle tissue, EV cargo, targeted assays; low	block accumulation	Low

Secretome / extracellular vesicles	EV abundance, CD9/CD63/CD81, EV-microRNA panels	intramuscular stress converted into liquid-biopsy signals	plasma or urine EVs; low	hours-24 h	Low
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Note: Evidence-base shorthand: Mod., moderate human evidence; Early + mech., early translational evidence supported by mechanistic studies; Explor., exploratory. CGM, continuous glucose monitoring; DBS, dried blood spot; MPO, myeloperoxidase; ROS/RNS, reactive oxygen/nitrogen species; eIF2alpha, eukaryotic initiation factor 2 alpha; ISR, integrated stress response; ATF4/ATF5, activating transcription factors 4 and 5; CHOP, C/EBP homologous protein; GDF15, growth differentiation factor 15; FGF21, fibroblast growth factor 21; TFEB/TFE3, transcription factors EB and E3; BNIP3L/NIX, BCL2 interacting protein 3-like/NIP3-like protein X; PINK1, PTEN-induced kinase 1; EV, extracellular vesicle. Field feasibility is a pragmatic estimate of how realistically a signal can be repeated in routine athlete monitoring. Most readouts below the redox-inflammatory layer remain research-stage and require longitudinal validation in well-phenotyped athlete cohorts [11–14,24,66,67,73,95,96].

Operationally, Table 4 shifts the question from whether a marker rises after hard training to whether that rise resolves on schedule for the individual athlete and context. The timing and feasibility cues are intended to guide when monitoring should simply be repeated, when deload should precede escalation, and when research-stage biology is unlikely to be useful in routine practice.

Table 4. Stage-linked monitoring logic linking load-recovery mismatch to biomarker-detectable maladaptation, with timing and field-feasibility cues.

Stage	Core molecular logic	Preferred readouts	Timing / feasibility	Monitoring use
Productive overload	Transient AMPK, catecholamine, and substrate stress that resolves on schedule	lactate or CGM trends; session CK/protein shifts	session-24 h / high	Expected rebound
Delayed immune-redox resolution	Acute-phase/complement persistence with emerging Nrf2/Keap1 buffering strain	serial proteomics, leukocyte ratios, exploratory oxidative panels	24-72 h / medium	Context review
Contractile-mitochondrial inefficiency	Reduced Ca ²⁺ sensitivity with ISR activation and rising mitochondrial stress tone	submaximal force tests, targeted blood markers, biopsy in research	48 h-block / medium	Explains prolonged weakness
Quality-control failure	TFEB/TFE3, BNIP3 and BNIP3L/NIX, PINK1/Parkin, and lysosomal-autophagic uncoupling	tissue markers, EV cargo, targeted assays	block-prolonged recovery / low	Mechanistic escalation

Systems phenotype of maladaptation	Endocrine drift plus	multimarker panel	weeks to	Deload +
	symptom-performance	plus phenotype	months /	clinical
	uncoupling across	anchors	medium	work-up
	tissues			

Note: The cascade is conceptual and stage-based, with overlap between adjacent states; it translates molecular direction into serial monitoring logic rather than a fixed temporal sequence or rigid diagnostic threshold. AMPK, AMP-activated protein kinase; CGM, continuous glucose monitoring; CK, creatine kinase; Nrf2/Keap1, nuclear factor erythroid 2-related factor 2/Kelch-like ECH-associated protein 1; ISR, integrated stress response; TFE3/TFEB, transcription factors EB and E3; BNIP3L/NIX, BCL2 interacting protein 3-like/NIP3-like protein X; PINK1, PTEN-induced kinase 1; EV, extracellular vesicles. Field feasibility refers to routine repeated use in applied monitoring rather than mechanistic importance [16,24,53–55,66,67,73].

5. Proteomics, Multi-Omics, and Molecular Integration as Next-Generation Biomarker Layers

5.1. Superiority of Multimarker Panels over Single Biomarkers

Emerging large-scale resources are beginning to show what a mature molecular reference frame for exercise actually looks like. Meta-analytic transcriptomic atlases, consortium-scale molecular mapping initiatives, and contemporary multi-omics resources now define conserved early-response modules, modality-dependent remodeling programs, and tissue-scale signatures across human skeletal muscle [18–21,98–103]. For maladaptation research, the implication is decisive: a biomarker panel should not be judged against a generic exercise response, but against the molecular direction expected for the specific loading paradigm, recovery window, tissue source, and athlete phenotype under study [20,21,97,100,101,104]. In practical terms, the near-term role of omics is not discovery alone, but calibration of the biological direction expected for a given load-recovery context [18–21,100,101].

The attraction of proteomics is not merely that it can measure more molecules. Its real value is that it captures coordinated biology. The FOR and NFOR studies already discussed suggest that maladaptation is expressed as a network-level shift involving acute phase proteins, innate immunity, complement activity, and broader immunometabolic processes [7,8]. That logic aligns well with more recent thinking in the OTS literature, where composite systems and complex-systems models have replaced the search for a single universal biomarker [3–5].

For a translational review intended for practitioners, this point is essential. Coaches do not need more isolated numbers; they need decision support, and any analytic layer introduced into monitoring should remain interpretable enough for clinicians and performance staff to understand why a profile is escalating rather than behave as an opaque classifier [6–8,16,27]. A useful biomarker panel should therefore satisfy four criteria: it should detect deviation from the athlete's own baseline, correlate with performance or recovery, remain interpretable across repeated measurements, and add value beyond symptom monitoring alone. Multi-omics strategies are attractive only to the extent that they satisfy those applied criteria [18–21,24,97,100].

Time resolution is a major reason omics may succeed where isolated biomarkers fail. A meta-analysis of 43 exercise transcriptomic studies showed that acute and long-term responses are transcriptionally distinct, with many genes displaying narrow time windows and strong modulation by modality, age, and other cohort characteristics [101]. For maladaptation research, this means an apparently inconsistent biomarker may simply have been sampled at the wrong biological moment. Omics should therefore be planned around kinetic questions: what rises immediately after overload, what remains elevated after 24–72 h, and what becomes chronically reset across a training block.

Equally important, overtraining biology should not be modeled as the mirror image of training adaptation. Comparative transcriptomic analyses show that decreased and increased skeletal-muscle

loading activate qualitatively different transcription-factor networks rather than simple inversions of the same program [105]. Epigenetic work further indicates that greater physiological and metabolic load can reshape DNA methylation together with genes linked to AMPK/MAPK signaling, VEGFA, NR4A1, NR4A3, and PPARGC1A [106]. Conceptual work on training-load oscillation and epigenetic plasticity likewise supports the view that maladaptation reflects altered remodeling dynamics rather than a simple linear excess of load [22]. This supports a systems view in which maladaptation reflects failed remodeling, not merely an exaggerated version of a normal acute response. The near-term challenge is therefore not to generate ever larger candidate lists, but to identify minimal multimarker combinations that remain stable, affordable, and decision-relevant in real athlete monitoring. Omics will not replace monitoring, but it will redefine its resolution.

5.2. Standardization, Phenotyping, and Study-Design Priorities

The fastest way to create noisy biomarker literature is to ignore pre-analytical control. Sampling time, fed or fasted status, matrix choice, recent exercise, menstrual or hormonal context, altitude or heat exposure, travel, assay platform, and recent infection can all distort interpretation [1,4,5,16,33]. This is one reason the field still lacks portable decision thresholds despite decades of interest. Recent athlete-biomarker guidance has also stressed the importance of analytical variation, meaningful minimal change, and the athlete's own reference range when serial values are used for workload management [16]. For emerging analytes such as extracellular vesicles or microRNA panels, those concerns are magnified rather than reduced [90,93,95].

A mature biomarker framework for training maladaptation must therefore be standardized at three levels: the athlete context, the sampling procedure, and the phenotype anchor [16,24,90,93,95]. Serial morning samples during defined training phases, repeated use of the same assay platform, matrix-specific handling protocols, and pairing of biomarker data with symptom and performance information should be considered minimal quality standards for future studies [7,8,16,24,90,93,95]. Without that discipline, even biologically important molecules will remain translationally weak [16,90,93].

Table 5. Pre-analytical and analytical priorities for future biomarker studies in training maladaptation.

Risk if ignored	Recommended standardization	Why it matters	Variable
False between-session differences	Use fixed morning or fixed post-exercise windows	Many signals are sharply time-sensitive after exercise	Sampling window
Mislabeling normal overload as pathology	Log training load, monotony, and competition density	Biomarkers reflect the preceding microcycle, not just chronic status	Recent load history
Confounded interpretation	Record core recovery stressors at each sampling point	Sleep, travel, illness, and energy availability modulate the same biology	Recovery environment
Reduced reproducibility	Document cycle phase, contraceptive use, or hormonal treatment where relevant	Endocrine signals vary With biological and pharmacological context	Hormonal context
Poor	Use the same matrix	Panel composition	Assay

comparability	and platform within a study or program	and absolute values vary by method	platform
Clinically weak conclusions	Pair sampling with symptoms and performance metrics	Biomarkers are meaningful only when tied to function	Phenotype anchor

Note: This checklist summarizes minimum pre-analytical and analytical controls for serial biomarker studies in athletes. Standardization is most informative when repeated within the same athlete, using the same sampling window, biospecimen matrix, and assay platform, and when interpreted alongside recent load, symptoms, performance, and recovery status [16,24,33,90,93,95].

These pre-analytical constraints explain much of the field's instability. Without fixed sampling windows, consistent matrix handling, and phenotype anchors, even biologically valid signals will continue to appear contradictory across cohorts [1,4,5,16,90,93]. External validity is further limited by small overload models, mixed training backgrounds, male-dominant samples, and the scarcity of longitudinal cohorts with clinically well-phenotyped OTS. Until larger datasets are available, the field should prioritize within-athlete tracking, staged escalation rules, and explicit capture of illness exposure, energy availability, hormonal context, and recovery environment over universal cut-offs.

6. Discussion: From Molecular Signals to Phenotype-Anchored Monitoring

The present synthesis extends beyond descriptive molecular mapping by proposing an integrated, phenotype-anchored framework that links mechanistic biology to staged monitoring and decision-making in athletes.

6.1. Answer to Q1: When Productive Overload Becomes Unresolved Maladaptation

The transition from productive overload to maladaptation is best identified by failed recovery, not by the peak acute response. Productive overload produces temporally appropriate perturbation, whereas unresolved maladaptation is characterized by recovery-day persistence of immune-redox activation, loss of athlete-like endocrine-metabolic patterning, and delayed return of function [1,2,4–6,9,10,16]. This distinction is biologically important because OTS is not simply the far-right tail of training load; it is a state in which the normal coupling between overload, repair, substrate restoration, and performance rebound begins to break down [5,11,15–17,65]. The field should therefore stop asking whether inflammation, hormonal drift, or mitochondrial stress occur after hard training; they do. It should instead ask whether these signals remain inappropriately open for the athlete's expected recovery window [4–6,16,23,24]. In practice, the timing of signal persistence is often more informative than the absolute height of the preceding excursion [16,23,24].

6.2. Answer to Q2: Which Biospecimens and Molecular Layers Are Most Informative

For near-term monitoring, the most informative first-line biospecimens remain serial blood measurements interpreted against phenotype: simple hematology, acute-phase or innate-immune proteins, and selected endocrine variables [3,6–8,10,16]; together with metabolic readouts linked to substrate use or contractile strain [35,107–111]. These layers are imperfect, but they are accessible, repeatable, and already connected to recovery duration or underperformance. Operationally, biomarker layers are most useful when tiered by deployment readiness: field-ready serial anchors, escalation assays used when first-line biology and phenotype remain discordant, and research-stage liquid biopsies or tissue-centered readouts. Proteomics becomes especially valuable when routine markers look equivocal because it captures coordinated immunometabolic directionality rather than isolated analytes [7,8,21,24,104]. By contrast, EV cargo, circulating microRNAs, and tissue-centered stress markers should currently be viewed as second-line or research-stage layers that help explain persistent or discordant cases rather than replace routine monitoring [21,66,90,93,95,97]. Where

under-fueling or impaired remodeling is suspected, nutritional correction and training-fuel realignment should precede deeper testing; targeted supplementation may support tissue adaptation, but it does not substitute for restoration of energy availability [33,48].

6.3. Answer to Q3: Why Single Biomarkers Fail and Why Multimarker Interpretation is Necessary

Single biomarkers fail repeatedly for three converging reasons: the phenotype is heterogeneous, the kinetics are time-sensitive, and the same analyte can be shifted by illness, low energy availability, sleep disruption, travel, sex-hormonal context, or the preceding microcycle [1,4–6,16,25,26,101]. A biologically meaningful marker can therefore be diagnostically weak when removed from timing and context. This is why the most robust framework is a phenotype-anchored multimarker model in which no analyte is interpreted alone and no value is read without baseline, recent load, symptoms, and recovery duration [3,5,6,16,24]. In practice, multimarker panels do not eliminate uncertainty; they refine it by showing whether the athlete is re-closing the recovery loop or drifting into persistent uncoupling [5,16,24].

A critical implication follows: much of the existing literature is not contradictory, but under-standardized. Small cohorts, single time points, broad labels such as fatigue, and poor control of recovery biology have produced a literature rich in mechanism yet weak in thresholds [4,16,23,90,93,112–114]. Future work should therefore privilege within-athlete serial designs over cross-sectional threshold hunting.

6.4. Answer to Q4: How Muscle-Centered Stress Programs Converge

The muscle-centered phenotype of maladaptation appears to emerge when excitation-contraction strain, redox signaling, mitochondrial ISR activation, and autophagy-mitophagy control remain engaged longer than recovery can absorb [11–14,56,57,66,68,71]. In that state, the athlete may present clinically with disproportionate weakness, altered submaximal force behavior, abnormal substrate selection, and persistent spillover of stress signals into circulation even before classical resting damage markers become dramatic [11–14,35]. This helps explain why blood-based monitoring remains necessary but incomplete: the decisive biology may originate in muscle, while the measurable field signal is only a downstream echo of incomplete organelle and contractile recovery [21,24,67,81,97].

Within this hierarchy, EV and microRNA layers have a plausible translational niche. They are best deployed when first-line monitoring remains abnormal or biologically discordant after adequate deload, and only under tightly standardized sampling, hemolysis control, matrix handling, and platform consistency [90,93,95]. Used in that way, they may function as second-line liquid biopsies of unresolved tissue stress rather than as front-line screening tools.

6.5. Answer to Q5: Study-Design Priorities and a Provisional Recovery Failure Index

The next generation of biomarker studies should be built around dense longitudinal phenotyping, fixed sampling windows, and preplanned escalation from accessible markers to deeper omics layers. Energy availability, chrono-nutritional timing, and hormonal context should be captured explicitly rather than treated as background noise [21–26,90–96].

As a practical synthesis, we propose a provisional Recovery Failure Index (RFI) as an implementation logic rather than a diagnostic threshold. Let phenotype deviation = P, immune-hematological persistence = I, endocrine-metabolic drift = E, and unresolved discordance requiring escalation = D; each domain is scored 0–2 points, giving $RFI_{raw} = P + I + E + D$ (range 0–8) and, when preferred for dashboards, $RFI\% = (RFI_{raw} / 8) \times 100$. The RFI is intended as a conceptual implementation tool to structure serial decision-making rather than as a validated diagnostic instrument. The score is intentionally unweighted to preserve interpretability and to avoid implying false precision before prospective calibration. Future work should test weighted, probabilistic, or

Bayesian variants only if they improve discrimination without sacrificing physiological transparency [27].

The score is intentionally serial and athlete-specific rather than cross-sectional. P can be graded from repeated performance loss, unusual fatigue, or delayed restoration of training tolerance; I from serial acute-phase or leukocyte-based abnormalities; E from loss of the athlete's expected hormonal or substrate-use profile; and D from persistent mismatch between symptoms, performance, and first-line biology despite deload and context correction. Importantly, the RFI should not be interpreted as a threshold-based classifier, but as a trajectory-sensitive index reflecting whether recovery processes are re-closing over time. A rising score suggests loss of recovery-loop closure, whereas a falling score during deload supports return toward adaptive overload.

For clinicians and performance staff, the operational message is straightforward: first, re-check context before interpreting biology; second, repeat first-line markers before escalating to deeper assays; third, treat persistent discordance as a trigger for broader clinical evaluation rather than as proof of OTS by itself [3,5,16,24].

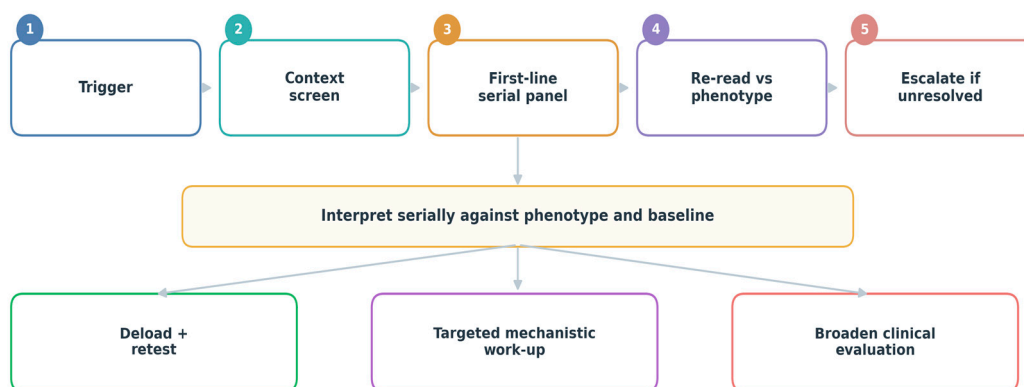


Figure 5. Phenotype-anchored workflow for biomarker-guided monitoring. The sequence proceeds from trigger to context screen, first-line serial panel, re-reading against phenotype, and escalation only when recovery remains unresolved. Management branches are deload and re-test when biology and function improve together, targeted mechanistic work-up when serial biology remains abnormal despite adequate recovery, and broader clinical evaluation when the pattern is discordant, severe, or suggestive of competing diagnoses. The workflow prioritizes serial interpretation against baseline rather than isolated abnormal values [16,24,33].

6.6. A Pragmatic Deployment Hierarchy for Real-World Monitoring

For applied monitoring, candidate biomarkers are most useful when separated by deployment readiness rather than placed in one undifferentiated list. Table 6 groups them into field-ready serial anchors, escalation assays used when first-line biology and phenotype remain discordant, and research-stage liquid biopsies or tissue-centered readouts. The purpose of this hierarchy is to reduce both false reassurance and overtesting: most athletes should be followed with repeated low-burden measures, whereas deeper omics layers should be reserved for persistent, atypical, or mechanistically unclear cases [16,21,24,90,93,97]. Interpretable analytic frameworks may help operationalize this hierarchy without turning multimarker monitoring into an opaque black box [27].

Table 6. Provisional deployment hierarchy for biomarker-guided monitoring of training maladaptation.

Tier	Primary purpose	Representative readouts	Practical trigger to advance
Field-ready serial anchors	Detect loss of performance expected	performance tests; symptom/recovery scores; simple	Advance when underperformance persists

	rebound in routine monitoring	hematology; acute-phase or innate-immune proteins; CK/lactate; selected endocrine-metabolic measures; CGM trends	or serial biology fails to normalize after deload/context correction
Escalation assays	Clarify discordance between phenotype and first-line markers	targeted proteomics; metabolite panels; submaximal force testing; targeted contractile surrogates	Advance when first-line data remain abnormal on repeat or a mechanistic explanation is needed
Research-stage / tissue-centered	Investigate unresolved cases; link circulating signals to muscle biology	EV cargo; microRNA panels; mitochondrial-stress candidates; biopsy-linked ISR/mitophagy readouts	Use in prospective studies or specialist work-ups, not for frontline screening

Note: The hierarchy is designed for serial interpretation, not one-off threshold hunting. Movement across tiers should be driven by persistent phenotype-biology discordance, delayed recovery, or competing diagnostic uncertainty rather than by isolated abnormal values [16,21,24,27,90,93]. CK, creatine kinase; CGM, continuous glucose monitoring; EV, extracellular vesicle; ISR, integrated stress response; GDF15, growth differentiation factor 15; FGF21, fibroblast growth factor 21.

Figure 6 integrates this hierarchy with the provisional RFI. In practice, 0-2 points suggest adaptive or resolving strain, 3-4 points justify context review and repeat first-line sampling, 5-6 points support deload plus targeted escalation, and 7-8 points justify broader clinical work-up for OTS mimics or entrenched maladaptation. The value of the RFI is not that it supplies a universal diagnostic threshold, but that it forces serial interpretation across phenotype, immune-hematological persistence, endocrine-metabolic drift, and unresolved biological discordance.

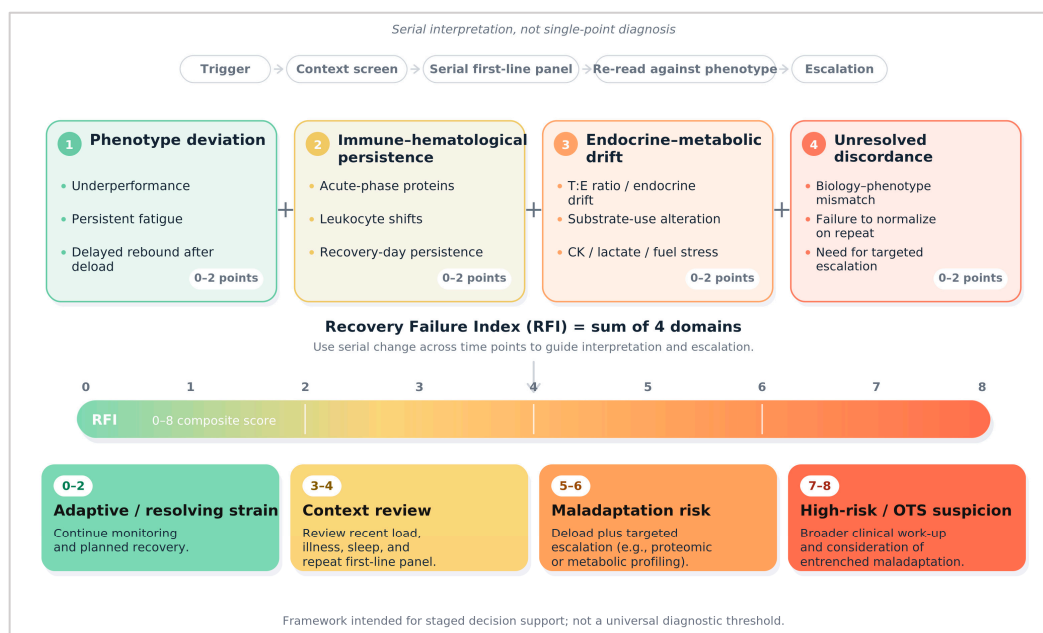


Figure 6. Provisional Recovery Failure Index (RFI) for staged decision support. Four domains-phenotype deviation, immune-hematological persistence, endocrine-metabolic drift, and unresolved discordance-are each scored 0-2 points, yielding an intentionally unweighted serial 0-8 composite index. The lower bands indicate pragmatic action zones: 0-2, adaptive or resolving strain; 3-4, context review and repeat first-line panel; 5-6, deload plus targeted escalation; and 7-8, broader clinical work-up and consideration of entrenched

maladaptation. The figure is intended as a serial decision aid rather than a universal diagnostic threshold [3,5,16,24,27].

6.7. Limitations

This review has several limitations. First, the evidence base remains heterogeneous in phenotype definition, sampling density, sport discipline, and translational depth, which limits direct comparison across studies. Second, clinically well-phenotyped longitudinal cohorts of true OTS remain rare, so several mechanistic inferences still rely on overload models, case studies, or pathway-focused preclinical work rather than large prospective athlete datasets. Third, many emerging omics, EV, and microRNA signals remain sensitive to pre-analytical variability, assay platform, and sampling timing, which constrains immediate field deployment. Fourth, the provisional RFI proposed here is a conceptual implementation framework and requires prospective validation, calibration against athlete-specific baselines, and testing across sex, sport type, and energy-availability contexts. Fifth, reverse causality remains a major unresolved problem: some molecular profiles may reflect pre-existing vulnerability, chronic low energy availability, illness burden, or constitutional differences in recovery capacity rather than maladaptation induced by training itself. Accordingly, the present synthesis should be read as a phenotype-anchored translational framework rather than as a source of universal diagnostic cut-offs.

7. Conclusions

Three conclusions emerge from the evidence reviewed here. Importantly, this review not only summarizes existing evidence but also reorganizes it into a systems-level, operational framework for the early detection of recovery failure.

First, overtraining is not a state of excess load, but a state of failed biological closure. Functional overreaching, non-functional overreaching, and overtraining syndrome are therefore best understood as progressively unresolved recovery biology, defined by convergence among immune, endocrine, metabolic, redox, and muscle-centered stress programs.

Second, the most useful biomarker architectures will be serial, phenotype-anchored, multimarker, and tiered by deployment readiness. A staged panel should begin with performance and symptom anchors plus accessible immune, endocrine, and metabolic readouts, escalate to targeted proteomic or metabolomic assays when recovery remains unresolved, and reserve EV or tissue-centered layers for refractory or research-stage questions. Such panels should privilege within-athlete baselines over cross-sectional thresholds, and the timing of signal persistence should usually carry more interpretive weight than the absolute magnitude of a single abnormal value. Within that logic, a provisional RFI offers a pragmatic way to summarize whether the recovery loop is closing across serial time points. At present, the RFI should be viewed as a translational framework to guide structured monitoring rather than as a standalone diagnostic tool.

Third, future studies should explicitly connect tissue biology to field monitoring. Longitudinal cohorts with dense sampling, explicit control of energy availability and sex-hormonal context, and muscle-to-blood integration are needed to validate practical tools such as the provisional RFI, test tiered escalation rules, and determine whether ISR, calcium-handling, mitophagy, and secretome signals improve decision-making in real athletes. The practical goal is earlier recognition of an open recovery loop, before prolonged maladaptation becomes entrenched.

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Abbreviations

AMPK	AMP-activated protein kinase
ATF4	activating transcription factor 4
CGM	continuous glucose monitoring
CHOP	C/EBP homologous protein
CK	creatine kinase
EV	extracellular vesicles
FOR	functional overreaching
HIIT	high-intensity interval training
ISR	integrated stress response
mTOR	mechanistic target of rapamycin
NF- κ B	nuclear factor kappa B
NFOR	non-functional overreaching
NOX2	NADPH oxidase 2
Nrf2	nuclear factor erythroid 2-related factor 2
OTS	overtraining syndrome
RFI	Recovery Failure Index
ROS	reactive oxygen species
SIRT1	sirtuin 1
TFEB	transcription factor EB
TFE3	transcription factor E3
UPRmt	mitochondrial unfolded protein response
VO ₂ max	maximal oxygen uptake

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