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Overtraining Syndrome and Hypothalamic-Pituitary-Adrenal Axis Dysfunction in Athletes: Mechanisms and Diagnostic Uncertainty - a narrative review

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Abstract

Background. Overtraining syndrome (OTS) is a maladaptive state arising from chronic imbalance between training/non-training stress and recovery, historically attributed to dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis. Despite decades of research, no validated biomarker or diagnostic threshold exists, and the directionality of HPA involvement remains contested.

Aim. To critically synthesise evidence on HPA-axis mechanisms in OTS, contrast them with competing pathophysiological hypotheses, and evaluate the diagnostic uncertainty limiting clinical recognition and management.

Materials and Methods. Narrative review of peer-reviewed English-language systematic reviews, cross-sectional and longitudinal cohort studies, case series, consensus statements, and mechanistic physiological studies addressing OTS, HPA-axis function, hormonal biomarkers, and related conditions in athletes.

Results. Basal cortisol and ACTH are generally unhelpful diagnostically, whereas stimulation-test responses (insulin tolerance test, two-bout exercise protocol) more consistently reveal blunted late hormonal reactivity in overtrained athletes, though findings remain inconsistent across cohorts. The testosterone-to-cortisol ratio, cytokine, glycogen, central-fatigue, autonomic, and gut-brain-axis hypotheses each explain fragments of the clinical picture without unifying explanatory power. Composite instruments (EROS-COMPLETE, EROS-SIMPLIFIED) show promising discrimination in small single-sex cohorts but lack external validation.

Conclusions. HPA-axis dysfunction in OTS is real but neither sufficient nor specific as a diagnostic criterion. Progress requires standardised stimulation-testing protocols, sex-inclusive cohorts, and multi-domain composite instruments rather than continued pursuit of a single biomarker.

Keywords: overtraining syndrome; hypothalamic-pituitary-adrenal axis; cortisol; testosterone-cortisol ratio; sports endocrinology; athlete monitoring; relative energy deficiency in sport

1. Introduction

Muscle fatigue and declining performance are familiar, self-limiting consequences of intense training when followed by adequate recovery; the same stimulus, applied without sufficient restitution, can instead precipitate a persistent and poorly understood clinical entity known as overtraining syndrome (OTS) [1]. OTS is most usefully conceived not as a discrete illness but as the far end of a continuum that begins with functional overreaching (FOR) — a short-term, planned performance decrement that resolves within days and is typically followed by supercompensation — passes through non-functional overreaching (NFOR), in which recovery is prolonged to weeks or months and accompanied by early psychological and neuroendocrine disturbance, and culminates in OTS itself, defined by the European College of Sport Science and American College of Sports Medicine (ECSS/ACSM) joint consensus statement as a state of performance decrement persisting for months to years, with more severe symptomatology and maladapted physiology across psychological, neurological, endocrine, and immunological systems, triggered by an additional stressor not explained by other disease [1,2].

Epidemiological estimates vary widely, reflecting both genuine differences between populations and the absence of standardised case definitions: lifetime prevalence figures ranging from 5% to 64% of athletes have been reported, with endurance sports characterised by high-volume, high-frequency training — swimming, triathlon, road cycling, and rowing — considered particularly high-risk [3-5]. One survey of elite male and female distance runners found that 64% and 66%, respectively, reported experiencing staleness at some point in their competitive careers [5]. Team sports appear to carry a lower burden than individual endurance disciplines, and a history of a prior OTS episode substantially increases the risk of recurrence [6].

The hypothalamic-pituitary-adrenal (HPA) axis has occupied a central position in OTS pathophysiology since the earliest systematic investigations, on the reasoning that chronic

training stress, superimposed on non-training stressors such as inadequate sleep, caloric restriction, and psychosocial strain, should progressively exhaust the neuroendocrine stress-response system in a manner analogous to other chronic-stress conditions [1,7]. Early case-based work identified a pattern of blunted pituitary hormonal responses to a hypoglycaemic challenge in overtrained endurance athletes, implicating a functional defect somewhere along the hypothalamic-pituitary axis rather than frank adrenal failure [8]. Subsequent decades of research have considerably refined this picture while simultaneously revealing how far the field remains from a validated diagnostic biomarker: basal cortisol and adrenocorticotropic hormone (ACTH) levels are now understood to be poor discriminators between overtrained and healthy athletes, whereas dynamic testing — particularly the insulin tolerance test (ITT) and two-bout exercise protocols — more consistently, though still imperfectly, exposes an impaired late hormonal response that may plausibly underlie the reduced time-to-fatigue characteristic of the syndrome [9,10].

At the same time, HPA-axis dysfunction has never stood as the sole explanatory framework for OTS. Competing and overlapping hypotheses — centred on glycogen depletion, central serotonergic and dopaminergic disturbance, cytokine-mediated inflammation, autonomic nervous system imbalance, oxidative stress, mitochondrial quality-control failure, and gut-brain axis dysregulation — each account for particular clinical features without achieving comprehensive explanatory power, and most authors now regard OTS as a genuinely multisystem, multifactorial condition rather than a single-axis disorder [1,11,12]. This conceptual plurality is compounded by substantial overlap between OTS and other conditions that share fatigue, mood disturbance, and hormonal suppression as core features, most notably relative energy deficiency in sport (RED-S) and major depressive disorder, both of which must be actively excluded, or their contribution weighed, before a diagnosis of OTS can be entertained [1,13].

The purpose of this narrative review is to critically synthesise the evidence specifically bearing on HPA-axis mechanisms in OTS: to describe what is known about basal and stimulated cortisol, ACTH, and related hormonal behaviour in overtrained athletes; to situate these findings within the broader landscape of competing neuroendocrine, metabolic, and inflammatory hypotheses; to examine the diagnostic tools that have been proposed to operationalise HPA-axis testing in clinical and sports-medicine practice; and to identify, with appropriate candour about the limitations of the underlying evidence, where the field's persistent diagnostic uncertainty originates and how it might be reduced. Throughout, the review adopts a comparative and critical stance, explicitly weighing studies against one another rather than treating each isolated finding as established fact, and it foregrounds methodological weaknesses — small sample sizes, near-total absence of female cohorts, heterogeneous case definitions, and the ethical impossibility of experimentally inducing true OTS — that qualify almost every conclusion that follows.

2. Materials and Methods

This article was conceived as a narrative review rather than a systematic review or meta-analysis, and no review protocol was registered; consequently, no formal risk-of-bias appraisal or quantitative pooling of effect sizes was undertaken, in keeping with the exploratory and mechanistically oriented nature of the topic. The literature considered comprised peer-reviewed, predominantly English-language publications addressing overtraining syndrome, functional and non-functional overreaching, hypothalamic-pituitary-adrenal axis physiology and dysfunction, hormonal and metabolic biomarkers in athletes, neuroinflammatory and gut-brain axis mechanisms relevant to exercise, relative energy deficiency in sport, and closely related endocrine and psychiatric conditions in athletic populations, spanning foundational

physiological studies, systematic reviews, cross-sectional and prospective cohort studies, case series, consensus and position statements, and mechanistic reviews of molecular and neuroendocrine pathways. Sources were selected for their direct relevance to the neuroendocrine and diagnostic dimensions of OTS and for their methodological standing within sports endocrinology and sports medicine; foundational mechanistic and historical papers were retained where they clarified the conceptual basis of current diagnostic practice, even where more recent studies existed, because the historical development of the HPA-axis hypothesis is itself part of the story this review seeks to tell. Evidence was grouped thematically into the following domains: definitions and the overreaching-to-overtraining continuum; competing pathophysiological hypotheses; hypothalamic-pituitary-adrenal axis physiology and its exercise-specific behaviour; basal hormonal markers; dynamic stimulation testing; the testosterone-to-cortisol and testosterone-to-estradiol ratios; composite diagnostic instruments (the EROS studies); systemic, immune, and gut-brain axis contributions; sex-specific and population-specific considerations; differential diagnosis, chiefly relative energy deficiency in sport and major depression; psychiatric and neurological manifestations; prevention and management; and future diagnostic directions. Because source selection and synthesis were, by design, narrative rather than systematic, the review may be subject to selection and emphasis bias, and its conclusions should be read as a clinically and mechanistically reasoned synthesis rather than a formally graded account of all available evidence.

3. The Overreaching-Overtraining Continuum: Definitions and Diagnostic Boundaries

The ECSS/ACSM joint consensus statement remains the most widely cited framework for classifying training-stress states, distinguishing functional overreaching, non-functional overreaching, and overtraining syndrome primarily by the duration of performance impairment and the completeness of recovery rather than by any biochemical threshold [1,2]. Functional overreaching represents an accumulation of training load producing a short-term performance decrement — typically resolving within days to a few weeks — that is frequently used deliberately within periodised training to elicit a subsequent supercompensatory improvement in performance [2,14]. Non-functional overreaching describes a state in which the performance decrement is more pronounced and the recovery period extends to weeks or occasionally months, accompanied by early psychological and neuroendocrine disturbance that nonetheless resolves with appropriate rest [1,2,14]. Overtraining syndrome, by contrast, denotes a chronic, maladaptive state in which performance impairment, psychological disturbance, and neuroendocrine, immunological, and metabolic dysfunction persist for months to years despite recovery attempts, and which by definition cannot be attributed to another identifiable disease process [1,2].

This tripartite framework, while conceptually useful, has been criticised for resting on a distinction — time to recovery — that is inherently retrospective and therefore of limited value for early clinical decision-making: a clinician cannot know, at the point an athlete first presents with fatigue and underperformance, whether the eventual course will be that of NFOR or established OTS [15]. A 2022 systematic review examining the OTS literature found that no published study had simultaneously demonstrated, with rigorous methodology, both an objectively documented prolonged performance decrement and a comprehensively characterised psychological symptom profile meeting predefined research criteria, exposing a fundamental evidentiary gap at the heart of the field [15]. This methodological fragility is compounded by decades of inconsistent terminology — "staleness," "burnout," "unexplained underperformance syndrome," and "paradoxical deconditioning syndrome" have all been used at various points to describe overlapping or identical clinical presentations — which has

impeded both the accumulation of a coherent evidence base and the clinical recognition of the condition [15,16].

Epidemiological estimates of OTS prevalence must be read against this definitional instability. Figures ranging from 5% to 64% across various athlete populations have been reported, and the true burden is almost certainly underestimated because many affected athletes never present for medical evaluation, or because their underperformance is attributed to inadequate motivation, technical deficiency, or unrelated illness rather than to a recognised syndrome [3,4]. Reported relapse rates of 80-90% within three years of apparent recovery further indicate that even where OTS is correctly identified and treated, the underlying vulnerability — whether constitutional, psychological, or related to persistent training culture — is rarely fully addressed [15,17].

Diagnosis, as codified in the ECSS/ACSM statement and reiterated across subsequent reviews, remains fundamentally one of exclusion: a documented decrement in sport-specific performance of clinically meaningful magnitude, persisting despite an adequate period of rest, in the absence of identifiable organic disease (thyroid dysfunction, anaemia, occult infection, cardiac disease) or another primary psychiatric disorder that would better explain the presentation [1,7,15]. No single laboratory test, imaging finding, or biomarker threshold is currently accepted as sufficient, alone, to confirm or exclude the diagnosis — a limitation that recurs throughout the mechanistic literature discussed in the sections that follow and that motivates much of the ongoing search for a validated composite instrument [1,15].

The practical consequence of this exclusion-based definition deserves emphasis, because it fundamentally shapes how every biomarker discussed later in this review should be interpreted. A test that shows an association with OTS in a cohort defined retrospectively, on the basis of prolonged performance decrement plus symptom persistence, cannot logically be validated as a prospective diagnostic instrument until it has been tested in athletes at risk before the diagnosis is otherwise apparent; nearly all of the biochemical and hormonal literature reviewed in Sections 6 through 10 was generated using precisely this retrospective design, meaning that even the most internally consistent findings remain, strictly speaking, associative rather than diagnostic in the prospective sense that clinicians would ultimately require [15,16].

4. Competing Pathophysiological Hypotheses: An Overview

Before examining HPA-axis mechanisms in detail, it is necessary to situate them within the broader field of competing explanatory frameworks, because much of the diagnostic uncertainty surrounding OTS arises precisely from the fact that several partially independent biological systems appear to be perturbed simultaneously, with no single system convincingly established as primary.

The glycogen depletion hypothesis holds that repeated high-intensity exercise without adequate carbohydrate replenishment produces chronic depletion of muscle and hepatic glycogen stores, which in turn impairs performance directly through substrate limitation and indirectly by increasing oxidation of branched-chain amino acids, thereby elevating the plasma tryptophan-to-branched-chain-amino-acid ratio and promoting central serotonergic activity [1,16,18]. This hypothesis is mechanistically coherent for exercise-induced fatigue in general but has not been reliably linked to glycogen status specifically in athletes diagnosed with OTS, some of whom present with normal glycogen stores, and long-term carbohydrate supplementation in animal models has attenuated but not abolished overtraining-induced performance decrements, suggesting the mechanism is contributory rather than sufficient [18,19].

The central fatigue hypothesis, closely related to the glycogen hypothesis, proposes that increased central serotonergic tone — driven by the altered tryptophan-to-branched-chain-

amino-acid transport ratio described above — produces fatigue, mood disturbance, and reduced motivation, while reciprocal suppression of dopaminergic signalling in the mesolimbic reward pathway contributes to anhedonia and reduced training drive [16,19]. Administration of selective serotonin reuptake inhibitors to athletes has been shown to reduce performance, and marathon runners supplemented with branched-chain amino acids have reported improved subjective energy and mental clarity, findings broadly consistent with the hypothesis, although the correlational nature of much of the supporting human data limits causal inference [19,20].

The glutamine hypothesis attributes the increased susceptibility to upper respiratory tract infection observed in overtrained athletes to chronic depletion of plasma glutamine, an amino acid central to lymphocyte and macrophage function; however, the relationship between circulating glutamine concentration and functional immune competence is inconsistent across studies, and glutamine supplementation has not reliably reduced infection rates in controlled trials, weakening confidence in a direct causal role [1,18,19].

The oxidative stress hypothesis holds that excessive training generates reactive oxygen species beyond the buffering capacity of endogenous antioxidant systems, producing lipid peroxidation, muscle damage, and a systemic inflammatory milieu that impairs recovery and contributes to fatigue; overtrained athletes have been shown in several small studies to exhibit higher resting markers of oxidative stress than matched controls, with further increases on subsequent exercise challenge, although whether oxidative stress is a cause or a consequence of the overtrained state remains unresolved [18,21].

The autonomic nervous system hypothesis proposes a shift toward relative parasympathetic dominance and reduced sympathetic reactivity in established OTS, manifesting as resting bradycardia, reduced heart rate variability responsiveness, and blunted catecholamine responses to stimuli; this framework has received support from studies of nocturnal urinary catecholamine excretion and heart rate variability but has also produced contradictory findings across studies employing different measurement protocols and training modalities [1,18,21].

The cytokine hypothesis, arguably the most comprehensive of the competing frameworks, proposes that repetitive musculoskeletal microtrauma combined with inadequate recovery time drives a chronic low-grade systemic inflammatory state, mediated principally by interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α), which subsequently act on the central nervous system to produce a pattern resembling "sickness behaviour" — anorexia, sleep disturbance, depressed mood, and reduced motivation — that closely mirrors the clinical presentation of OTS [16,22]. This hypothesis has the attractive feature of linking peripheral tissue damage directly to the neuropsychiatric symptomatology of OTS via a well-characterised biological pathway, and it also provides a mechanistic bridge to the HPA-axis hypothesis, because pro-inflammatory cytokines are potent activators of the HPA axis at the hypothalamic and pituitary level, while glucocorticoids in turn exert negative feedback on cytokine production — establishing a bidirectional relationship that several authors regard as central to the pathophysiology of the syndrome [16,22,23].

None of these hypotheses, taken alone, accounts for the full symptomatic breadth of OTS, and the field has progressively moved toward viewing the condition as arising from the convergent failure of multiple interacting regulatory systems rather than from dysfunction of any single axis — a conceptual shift that has direct implications for diagnostic strategy, discussed further in Section 14 [12,24]. The HPA axis, to which the remainder of this review is chiefly devoted, occupies a privileged position within this multisystem model both because of its central role in coordinating the physiological stress response and because it is mechanistically linked, via cortisol, to nearly every other hypothesis outlined above.

It is worth making explicit exactly how these frameworks intersect, since the literature rarely states this plainly. Glucocorticoid excess or dysregulation can itself alter substrate utilisation and hasten glycogen depletion, providing a mechanistic route by which HPA-axis disturbance could aggravate, rather than merely accompany, the glycogen-depletion and central-fatigue pathways; conversely, chronic glycogen insufficiency has itself been proposed as a chronic activator of the HPA axis, since hypoglycaemia is among the most potent physiological stimuli for cortisol and ACTH release, as demonstrated directly by the mechanism underlying the insulin tolerance test discussed in Section 7 [9,18]. Similarly, the cytokine hypothesis and the HPA-axis hypothesis are not merely adjacent but functionally interdependent: cortisol is the principal endogenous anti-inflammatory hormone, and a blunted late-phase cortisol response of the kind documented in overtrained athletes could plausibly permit unchecked or prolonged cytokine signalling that would, in a healthy axis, be actively suppressed, offering one plausible explanation for why some but not all cohorts of overtrained athletes demonstrate elevated inflammatory markers [16,22,24]. Because these hypotheses are mechanistically entangled rather than mutually exclusive, attempts to rank them by relative explanatory importance may be somewhat misguided; a more productive framing, adopted implicitly by several of the sources reviewed here, treats OTS as a state in which multiple normally self-limiting and mutually regulating systems lose their capacity for reciprocal restraint, producing a self-perpetuating rather than a linearly causal pathological process [12,24].

5. Hypothalamic-Pituitary-Adrenal Axis Physiology in the Context of Exercise

The HPA axis is the principal neuroendocrine system mediating the physiological response to both physical and psychological stress. Under conditions of acute stress, the hypothalamus releases corticotropin-releasing hormone (CRH), which stimulates the anterior pituitary to secrete adrenocorticotropic hormone (ACTH); ACTH in turn stimulates the adrenal cortex to synthesise and release cortisol, which mobilises energy substrates, modulates cardiovascular tone, and exerts negative feedback at both the hypothalamic and pituitary level to terminate the stress response once the triggering stimulus has resolved [12,23]. In the acute setting, this cascade is adaptive: cortisol and catecholamine release during exercise increase the availability of metabolic substrates, protect against excessive immune activation, and help maintain vascular integrity, while sustained elevation in the hours following exercise is thought to prepare the organism for a subsequent training stimulus [25].

Exercise itself is a potent, graded activator of the HPA axis. In humans, endurance exercise performed above approximately 60% of maximal oxygen uptake reliably activates the HPA axis, with a roughly linear relationship observed between exercise intensity and the magnitude of plasma ACTH and cortisol elevation above this threshold; below this intensity, HPA-axis activation may still occur but typically requires a longer duration of exercise, on the order of 90 minutes or more at around 40% of maximal oxygen uptake, to become measurable [26]. Following a single bout of intense or prolonged endurance exercise, cortisol concentrations generally return to baseline within roughly 150 minutes, and the magnitude of the subsequent HPA response is further modulated by recovery interval: in male elite endurance athletes, a repeated bout of prolonged exercise elicited a more pronounced ACTH and cortisol response when the preceding bout had occurred three hours earlier rather than six hours earlier, indicating that even in the acute setting, incomplete recovery time measurably sensitises the axis [26].

With regular, well-periodised training, athletes appear to develop a chronic adaptation characterised by mildly elevated resting evening ACTH and cortisol concentrations relative to sedentary individuals and to less intensively trained runners, alongside a somewhat blunted cortisol response to a subsequent acute exercise bout relative to what would be predicted from the intensity of exercise alone — a pattern interpreted as reflecting the development of adaptive

mechanisms to a chronically repeated stressor [26]. This is a critical point for interpreting subsequent OTS research, because it establishes that the "normal" trained-athlete HPA phenotype already differs substantially from that of the sedentary population, meaning that comparisons of overtrained athletes against untrained controls, rather than against matched healthy athletes, risk conflating the effects of chronic training per se with the effects of OTS specifically.

Distinct types of exercise appear to engage the HPA axis, catecholaminergic system, and associated inflammatory pathways somewhat differently. A single bout of endurance exercise reliably produces cortisol elevation together with activation of the locus coeruleus-noradrenergic system and increases in growth hormone (GH) and prolactin, whereas regular endurance training is associated with a state of moderate chronic HPA activation — elevated baseline interleukin-6 and cortisol — alongside a comparatively moderated acute cortisol response, consistent with the development of adaptive mechanisms described above [25]. High-intensity interval exercise (HIIE) similarly stimulates the HPA axis acutely, though in individuals with obesity, a single bout of HIIE has, somewhat paradoxically, been associated with an acute decrease in cortisol relative to isoenergetic resistance training, a finding the original authors attributed to the already-elevated baseline cortisol concentrations typical of obesity; with repeated HIIE training, basal cortisol concentrations tend to decrease, while catecholamine responses become blunted relative to the response observed after a single bout [25]. Resistance exercise produces a more variable HPA response that depends heavily on the intensity, volume, and muscle mass involved, with the largest ACTH and cortisol elevations observed for protocols combining large muscle groups, moderate-to-high loads, high total volume, and short rest intervals between sets; regular resistance training, by contrast, is generally characterised by reduced resting cortisol concentrations, distinguishing its chronic adaptation pattern from that of endurance training [25].

These modality-specific differences matter directly for the interpretation of OTS research, because much of the foundational literature on HPA-axis dysfunction in OTS derives from endurance-sport cohorts (distance running, triathlon, cycling, rowing), and it remains unclear how well these findings generalise to strength-based or mixed-modality athletes, an issue explicitly acknowledged as a limitation in several of the primary studies discussed below [9,10,24]. This uncertainty is compounded by the observation, discussed further in Section 10, that catecholamine responses in OTS appear to differ qualitatively between endurance and resistance-trained cohorts, suggesting that the modality-specific adaptation patterns documented in healthy athletes may translate into modality-specific patterns of dysfunction once overtraining is established, rather than a single, uniform "overtrained phenotype" applicable across all sports [27].

A further physiological nuance relevant to the interpretation of subsequent sections concerns diurnal and circadian variation in HPA-axis output, which interacts substantially with training timing. Cortisol secretion follows a pronounced circadian rhythm, peaking shortly after waking and declining progressively through the day, and training sessions conducted at different times relative to this rhythm may therefore be superimposed upon quite different baseline hormonal states, complicating comparison of studies that did not standardise sampling time relative to both waking and training session timing [9,26]. The EROS-HPA axis study's use of salivary cortisol rhythm sampling, discussed in Section 7, represents one of the few attempts in the reviewed literature to explicitly account for this circadian dimension rather than relying solely on single-timepoint venous sampling, and the fact that even this more circadian-sensitive approach produced a statistically significant group difference at only one of the sampled

timepoints (30 minutes post-awakening) illustrates just how much residual noise this dimension introduces into cross-sectional hormonal comparisons [9].

6. Basal Hormonal Markers: A Consistently Disappointing Diagnostic Yield

The earliest and most intuitively appealing approach to detecting HPA-axis dysfunction in OTS has been the measurement of basal, resting hormone concentrations — principally cortisol, ACTH, and testosterone — on the assumption that a chronically stressed or exhausted axis should manifest as an altered resting hormonal profile detectable on a single morning blood draw. The accumulated evidence, however, is strikingly consistent in showing that this approach largely fails.

A systematic review of the hormonal aspects of OTS, examining 38 studies meeting inclusion criteria after an extensive search of the literature, found that basal cortisol and ACTH levels were similar between OTS-affected athletes, healthy athletes, and — where measured — sedentary controls in the overwhelming majority of studies: among 17 studies comparing basal hormone levels between affected and healthy athletes, normal levels were disclosed for essentially all parameters and all hormones, with the exceptions of plasma catecholamines (elevated in one of two studies) and insulin-like growth factor-binding protein-3 (IGFBP-3, reduced in one study) [10]. When basal levels measured in previously affected athletes were pooled together with resting levels measured in athletes experimentally induced into a non-functional overreaching state via an overload training program — a combined analysis spanning up to 24 studies for some hormones — cortisol remained normal in 72.7% of comparisons, total testosterone in 64.7%, and ACTH in 80.0%, with the single partial exception of the testosterone-to-cortisol ratio, which was reduced in 41.7% of comparisons but normal in 50.0% [10]. This systematic review concluded unambiguously that basal hormone levels are not a good predictor of OTS, in sharp contrast to the more promising, though still imperfect, performance of stimulated hormone responses discussed in the following section [10].

The EROS-BASAL study, a cross-sectional comparison of 51 men (14 with OTS, 25 healthy athletes, 12 sedentary controls), reinforced this picture with more granular detail, applying rigorous inclusion criteria designed to ensure that the OTS group genuinely represented established syndrome rather than transient overreaching, and simultaneously including sedentary controls to distinguish dysfunction attributable to OTS from the mere loss of exercise-related adaptive changes [24]. Basal total testosterone was found to be significantly lower in OTS-affected athletes than in healthy athletes, but critically, basal testosterone in the OTS group did not differ significantly from that of sedentary non-athletes — a pattern interpreted by the authors as reflecting loss of a positive training adaptation (elevated testosterone in healthy athletes) rather than an overt pathological suppression below the normal population range [24]. Basal estradiol, by contrast, was significantly higher in OTS-affected athletes than in both healthy athletes and sedentary controls, and the testosterone-to-estradiol ratio was correspondingly reduced by roughly half relative to both comparator groups, a finding the authors interpreted as reflecting pathologically increased peripheral aromatase activity converting testosterone to estradiol rather than a purely central gonadotrophic abnormality, since gonadotrophic drive (inferred from the maintained testosterone response pattern) appeared preserved [24]. Total nocturnal urinary catecholamines were significantly elevated in OTS relative to healthy athletes, and creatine kinase, lactate, and total catecholamines were likewise elevated in OTS compared with healthy athletes, while classical inflammatory markers — erythrocyte sedimentation rate, C-reactive protein, ferritin — showed no significant differences between groups, leading the authors to conclude that OTS in this male cohort was not accompanied by frank clinical inflammation as measured by these standard markers, notwithstanding the mechanistic plausibility of the cytokine hypothesis [24].

Importantly, resting cortisol levels have consistently failed to differentiate athletes with and without NFOR or OTS across the broader literature, and reported testosterone responses have been frankly contradictory between studies, some showing reductions and others no change [16,28]. Several authors have proposed that a decreased testosterone-to-cortisol ratio, particularly a fall exceeding 30% from an individual's own baseline, might serve as a diagnostic indicator of insufficient recovery, but this threshold represents the physiological strain of training load itself rather than a validated marker of pathological maladaptation specifically, and its utility as a standalone diagnostic criterion has not been supported by subsequent literature [16,29]. A comprehensive narrative review of the testosterone-cortisol ratio (TCR) across sports endocrinology similarly concluded that basal TCR is influenced by such a wide array of factors — sex, competition anxiety, sport type, training volume, time of day, and menstrual phase — that a single absolute threshold cannot be reliably established, and that serial, within-athlete monitoring of the ratio over time is likely to be more informative than any single cross-sectional value [29].

Taken together, the basal-marker literature illustrates a recurring methodological lesson that applies well beyond the specific hormones discussed here: a single cross-sectional measurement, however biologically plausible its rationale, cannot reliably capture a dynamic regulatory failure, particularly when the "normal" reference range against which an athlete is compared is itself poorly defined and substantially modified by training status, sport type, sex, and time of measurement. This lesson motivates the shift toward dynamic stimulation testing described in the following section, and it also explains why composite and ratio-based approaches, discussed in Sections 8 through 10, have generally outperformed single basal measurements.

7. Dynamic Stimulation Testing: A More Promising but Still Imperfect Approach

Given the disappointing performance of basal hormone measurement, research attention has increasingly focused on dynamic stimulation protocols designed to probe the functional reserve of the HPA axis under standardised, exercise-independent challenge, on the theoretical basis that basal secretion may be preserved even when the capacity to mount an appropriately amplified response to acute stress is impaired — a pattern recognised in other endocrine conditions, such as relative adrenal insufficiency, in which basal cortisol can appear normal despite a blunted response to ACTH stimulation [9].

7.1 The insulin tolerance test

The insulin tolerance test (ITT), in which a standardised dose of intravenous insulin is administered to induce controlled hypoglycaemia as a potent, exercise-independent activator of the HPA and somatotropic axes, has been used as a gold-standard probe of the integrity of the entire hypothalamic-pituitary-adrenal axis, since a normal response requires unimpaired function at every level — hypothalamus, pituitary, and adrenal cortex — and any abnormality detected, given a normal adrenal response to a separate direct cosyntropin stimulation test, can be localised centrally rather than to the adrenal gland itself [9].

The EROS-HPA axis study applied this logic directly, comparing cortisol, ACTH, and salivary cortisol rhythm responses across OTS-affected athletes (n=14), healthy athletes (n=25), and non-active sedentary controls (n=12) [9]. Cortisol response to a 250 μg cosyntropin stimulation test — directly assessing adrenal reserve — was similar across all three groups, confirming that the adrenal gland itself responds normally to synthetic ACTH in OTS and that any dysfunction identified must lie upstream, at the hypothalamic or pituitary level [9]. During the ITT, mean cortisol response 30 minutes after hypoglycaemia was significantly higher in healthy athletes (21.7 μg/dL, increase of 9.2 μg/dL from baseline) compared with both OTS-affected athletes

(17.9 μg/dL, increase of 6.3 μg/dL) and sedentary controls (16.9 μg/dL, increase of 6.0 μg/dL); notably, the OTS group's response did not differ significantly from that of sedentary non-athletes, again suggesting loss of an athletic adaptation rather than a pathological deficit relative to the general population [9]. Median ACTH response 30 minutes after hypoglycaemia followed a similar pattern, being significantly higher in healthy athletes (59.9 pg/mL, increase of 45.1 pg/mL) than in OTS-affected athletes (30.3 pg/mL, increase of 9.7 pg/mL) or sedentary controls (51.4 pg/mL, increase of 38.0 pg/mL) [9]. The authors proposed provisional diagnostic cutoffs based on these data — a cortisol response below 17.0 μg/dL or an ACTH response below 35 pg/mL suggesting a positive predictive value for OTS, while a cortisol response above 20.5 μg/dL or an ACTH increase above 70 pg/mL carried a high negative predictive value for excluding the diagnosis — although these thresholds derive from a single cohort of 51 male participants and require independent replication before clinical adoption [9].

Salivary cortisol rhythm assessment in the same cohort revealed that cortisol concentration 30 minutes after awakening was significantly higher in healthy athletes (500 ng/dL) than in both OTS-affected athletes (323 ng/dL) and sedentary controls (393 ng/dL), while the cortisol awakening response overall, though numerically higher in the healthy athlete group, did not reach statistical significance across groups, likely reflecting high inter-individual variability in this measure [9]. The authors interpreted the combination of findings as indicating that healthy athletes develop a form of "hormonal conditioning" — an enhanced, exaggerated HPA-axis response to standardised stressors — that is at least partially lost in OTS, such that the overtrained athlete's neuroendocrine profile comes to resemble that of an untrained, sedentary individual rather than exhibiting frank pathological suppression below population norms [9,24]. This interpretation, while internally consistent across the EROS-HPA and EROS-BASAL datasets, rests on a single research group's cohort and awaits confirmation by independent investigators using comparable stimulation protocols.

It is worth pausing on the clinical practicality of the ITT itself, since this bears directly on whether the promising research findings summarised above are likely to translate into routine clinical use. The ITT requires deliberate induction of hypoglycaemia under close medical supervision, is contraindicated in individuals with cardiovascular or seizure disorders, and demands considerable resources — physician presence, intravenous access, glucose rescue availability — that are simply not compatible with routine screening of large athlete populations, or with primary-care or team-physician settings where the majority of athletes with suspected OTS are likely to first present [9]. This practical limitation means that, even if the provisional diagnostic cutoffs proposed by the EROS-HPA axis study were to be externally replicated, the test's clinical utility would probably remain confined to specialist referral centres for diagnostically ambiguous cases, rather than functioning as a frontline screening tool — a consideration that strengthens, rather than weakens, the case for the composite and non-invasive monitoring strategies discussed in Sections 10 and 16.

7.2 The two-bout exercise protocol

An alternative, arguably more ecologically valid approach to stimulation testing involves repeating a standardised maximal exercise challenge twice within the same day, separated by several hours of rest, on the theoretical basis that a healthy HPA axis should be able to mount a comparable — or even an exaggerated — hormonal response to the second bout, whereas a maladapted axis, whether through early sensitisation (as proposed in NFOR) or eventual exhaustion (as proposed in established OTS), should show a characteristically altered pattern [12]. Early work using this protocol in overtrained endurance athletes found blunted ACTH and cortisol responses to induced hypoglycaemia relative to healthy controls, an observation

frequently cited as among the earliest direct evidence of HPA-axis dysfunction in the syndrome [12].

A subsequent refinement of the two-bout protocol proposed that the three states along the overreaching-to-overtraining continuum might be distinguished by qualitatively different response patterns to the repeated challenge: functional overreaching characterised by a somewhat diminished hormonal response to the second bout relative to the first (representing early, still-reversible adaptation), non-functional overreaching characterised paradoxically by an exaggerated hormonal response to the second bout (interpreted as reflecting hypersensitisation of the axis prior to eventual exhaustion), and established OTS characterised by an extremely exaggerated response to the first bout but an essentially absent response to the second bout, consistent with a genuinely exhausted rather than merely sensitised system [12]. If replicated, this proposed triphasic pattern would represent a meaningfully more nuanced model than a simple linear progression from normal to blunted responsiveness, but the original study distinguishing NFOR from OTS using this protocol defined OTS retrospectively and somewhat arbitrarily as symptom persistence exceeding one year, introducing a circularity that limits the strength of the conclusion that can be drawn regarding causal hormonal mechanisms [12].

This triphasic model, while not directly validated against the EROS-HPA axis findings described above, is at least broadly compatible with them: both frameworks converge on the idea that established OTS is characterised by a blunted, rather than exaggerated, response to a second or delayed challenge, while differing chiefly in whether an intermediate hypersensitisation phase genuinely exists as a distinct, detectable physiological state (as proposed by the two-bout protocol) or whether the transition from healthy adaptation to established dysfunction is better characterised as a graded loss of "hormonal conditioning" without a clearly demarcated intermediate phase (as implied by the EROS dataset). Distinguishing between these two models would require a genuinely longitudinal study following individual athletes as they transition from health through NFOR to established OTS, using a single standardised stimulation protocol throughout — a study design that, to our knowledge, has not yet been conducted, representing one of the clearest unmet needs in this literature.

7.3 Reconciling basal and stimulated findings

Taken together, the stimulation-testing literature supports a coherent, if still provisional, model: basal HPA-axis function is largely preserved in OTS, but the capacity to mount an amplified, sustained hormonal response to an acute physiological challenge is impaired, particularly in the later phase of the response curve (30 minutes post-stimulus rather than the immediate peak) [9,12]. This temporal pattern — normal early response, blunted late response — has been proposed as a plausible mechanistic explanation for one of the most clinically recognisable features of OTS: the tendency of affected athletes to begin a training session or competitive effort at an apparently normal pace, only to experience a disproportionately early and severe decline in performance as the session progresses, consistent with an inability to sustain the hormonal support required for prolonged effort [9]. However, this explanatory model, while elegant, rests on a relatively small evidence base concentrated within a small number of research groups, predominantly examining male athletes practising combined endurance and resistance training, and it has not yet been tested prospectively as a predictive tool in athletes at risk of developing OTS rather than retrospectively in those already diagnosed [9,24].

8. The Testosterone-to-Cortisol and Testosterone-to-Estradiol Ratios

Because basal single-hormone measurements have performed so poorly as diagnostic markers, considerable attention has been directed toward composite ratios intended to capture the balance between anabolic and catabolic hormonal signalling, on the reasoning that the relationship between two interacting hormones might prove more informative than either measured in isolation.

The testosterone-to-cortisol ratio (TCR) has the longest history of use in this context, reflecting the conceptually appealing idea that testosterone, as the principal anabolic hormone promoting muscle protein synthesis, and cortisol, as the principal catabolic hormone promoting protein breakdown, together represent a net anabolic-catabolic balance whose disruption toward a catabolic state might signal insufficient recovery or impending overtraining [29]. During acute exercise, both testosterone and cortisol typically rise together in the initial phase, but as exercise continues, elevated cortisol progressively suppresses testosterone synthesis, producing transient TCR reductions of up to 30% in untrained individuals during the first days of an intensified training block, with these acute fluctuations typically normalising as training continues in the absence of true overtraining [29]. In well-adapted, chronically trained athletes, the TCR appears to stabilise at a somewhat elevated level relative to untrained individuals, representing a positive training adaptation, whereas athletes exposed to genuinely excessive training loads without adequate recovery time may show a persistently reduced ratio, reflecting a durable shift toward a catabolic hormonal milieu [29].

However, the TCR is subject to substantial confounding. Its value depends heavily on the duration and intensity of the preceding exercise bout, with cortisol rising disproportionately relative to testosterone during exercise lasting beyond approximately 120 minutes regardless of intensity, while shorter-duration exercise of under 60 minutes produces comparatively modest cortisol elevation irrespective of intensity [29]. The ratio also differs by sex: in one study of elite endurance athletes monitored across an entire competitive season, cortisol increased significantly across the season in women but not in men, despite similar mean testosterone and TCR values between sexes, while in a separate cohort of amateur rowers, a reduced TCR was associated with poorer race performance specifically in female but not male athletes, indicating that sex modifies not merely the absolute values but potentially the clinical meaning of the ratio [29]. Competition anxiety introduces a further layer of complexity: official competition settings produce a more pronounced cortisol rise, and consequently a greater reduction in the TCR, than simulated competition of identical physical demand, yet athletes with higher pre-competition cortisol have in some studies performed better than those with lower pre-competition cortisol, suggesting that a reduced TCR in this specific context may reflect adaptive competitive arousal rather than pathological overreaching [29]. Given this multifactorial influence, the consensus emerging from recent reviews is that a single absolute TCR threshold cannot be meaningfully applied across athletes, sports, or sexes, and that serial, within-athlete tracking of the ratio relative to an individual's own established baseline, interpreted alongside training load and competitive context, is likely to carry considerably more diagnostic value than any cross-sectional comparison against population norms [29].

An additional practical consideration in interpreting the TCR literature is measurement timing relative to circadian rhythm and recent training load, since both testosterone and cortisol independently follow circadian patterns that are not necessarily synchronised, and comparison of TCR values obtained at different times of day, or following training sessions of differing duration and intensity, risks introducing an artefactual signal entirely unrelated to genuine training-load imbalance [29]. This methodological caveat applies with particular force to studies comparing TCR across different sports or competitive levels without standardising

sample collection protocols, and it substantially limits the ability to compare absolute TCR values reported across the heterogeneous studies synthesised in the broader OTS literature.

An alternative approach, the testosterone-to-estradiol (T:E) ratio, was specifically proposed by the EROS research group as outperforming either testosterone or estradiol measured alone, and outperforming the TCR, as a predictor of both metabolic and psychological status in athletes [24]. As described in Section 6, the EROS-BASAL study found the T:E ratio reduced by approximately 43% (from a mean of 20.8 in healthy athletes to 10.8 in OTS-affected athletes) independently of other measured variables, with a proposed clinical cutoff of approximately 13.7:1 (for testosterone expressed in ng/dL and estradiol in pg/mL) distinguishing normal from pathologically reduced ratios [24,30]. A subsequent joint analysis combining data across multiple arms of the EROS study reinforced this finding, demonstrating that the T:E ratio, rather than testosterone or estradiol individually, independently predicted the measured-to-predicted basal metabolic rate ratio and the chest-to-waist circumference ratio, while fatigue levels on the Profile of Mood States (POMS) questionnaire were inversely correlated with the T:E ratio [30]. The authors proposed a mechanistic rationale distinguishing two categories of estradiol elevation: a physiological increase accompanying a concurrent testosterone increase (in which the T:E ratio remains preserved and the elevated estradiol confers genuine benefit for libido, bone density, and body composition), versus a pathological increase driven by exacerbated peripheral aromatase activity converting testosterone to estradiol in the absence of increased gonadotrophic drive (in which the T:E ratio falls and the hormonal environment becomes net anti-anabolic) [24,30]. While internally coherent and mechanistically plausible, this framework again derives predominantly from a single research group's male-only cohort, and independent replication in other athlete populations, including women, has not yet been reported in the literature reviewed here.

9. Growth Hormone, Prolactin, and Insulin-Like Growth Factor Responses

Beyond the corticotropic axis itself, disturbances in growth hormone (GH), prolactin, and insulin-like growth factor-1 (IGF-1) secretion have been repeatedly implicated in OTS, and because GH and prolactin are co-released alongside ACTH and cortisol during the ITT, their behaviour provides an additional, mechanistically related window onto pituitary function in overtrained athletes.

In the EROS-STRESS component of the EROS study, basal GH was found to be significantly lower in OTS-affected athletes than in healthy athletes, as was basal prolactin, while both GH and prolactin responses to induced hypoglycaemia during the ITT were markedly blunted in the OTS group relative to healthy athletes at both the early (during hypoglycaemia) and late (30 minutes post-hypoglycaemia) timepoints, with the late GH response showing a particularly pronounced reduction [24,31]. This pattern of blunted somatotropic and lactotropic responses alongside blunted corticotropic responses to the same standardised stimulus supports the broader "hormonal conditioning loss" hypothesis outlined in Section 7: rather than each axis being independently and specifically disrupted, a more parsimonious interpretation is that OTS represents a generalised reduction in hypothalamic-pituitary responsiveness to acute physiological challenge, engaging multiple anterior pituitary hormone axes in parallel rather than selectively targeting the corticotropic axis alone [24,31].

A subsequent correlational analysis pooling data across the EROS study's constituent arms reinforced this interpretation directly: early and late cortisol, GH, and prolactin responses to the ITT were found to be strongly and positively intercorrelated with one another (correlation coefficients ranging from approximately 0.69 to 0.80 across the various hormone pairs), such that the magnitude of an individual athlete's hormonal response to stimulation appeared largely

uniform across all three hormones examined rather than dissociated, indicating — in the interpretation offered by the study's authors — that hypothalamic-pituitary responsiveness to stimulation is essentially a diffuse, non-axis-specific phenomenon in this population rather than a pattern in which certain axes are selectively spared or selectively impaired [30]. These hormonal responses were, in turn, positively correlated with self-reported vigour, fat oxidation, measured-to-predicted basal metabolic rate, and muscle mass, and inversely correlated with fatigue and body fat percentage, suggesting that whatever underlying process governs hypothalamic-pituitary reactivity to stimulation, it is broadly linked to the overall metabolic and psychological phenotype that distinguishes overtrained from healthy athletes, though the correlational design of this analysis precludes any conclusion regarding the direction of causality [30].

Basal IGF-1, by contrast, did not differ significantly between OTS-affected and healthy athletes in the EROS cohort, nor did the testosterone-to-cortisol ratio, both parameters having been proposed in earlier literature as candidate OTS biomarkers but failing to demonstrate discriminative value in this particular dataset, a negative finding the authors explicitly noted as contradicting some prior expectations [30]. This divergence between hormones that do and do not discriminate OTS from health — cortisol and GH stimulated responses discriminating, basal IGF-1 and the TCR not discriminating, at least in this cohort — illustrates the broader difficulty of the field: the pattern of abnormality appears to depend substantially on which specific hormone, which specific testing modality (basal versus stimulated), and which specific study population is examined, precluding confident generalisation from any single dataset to the OTS population as a whole.

This somatotropic dimension of OTS also carries plausible downstream metabolic consequences that extend well beyond the hormonal profile itself. Because GH and IGF-1 are central regulators of lipolysis, protein synthesis, and connective tissue repair, a chronically blunted somatotropic response to stimulation, if genuinely present and sustained over months, could plausibly contribute to the delayed musculoskeletal recovery, reduced lean mass accrual, and heightened injury susceptibility anecdotally described in athletes with established OTS, even though a direct, prospective link between blunted stimulated GH response and objectively measured injury or recovery outcomes has not, to our knowledge, been demonstrated within the sources reviewed here. This represents a further area in which mechanistic plausibility currently outstrips direct empirical confirmation, a pattern that recurs throughout this review and is discussed further in Section 18.

10. Composite Diagnostic Instruments: The EROS Scoring Systems

Recognising that no single biomarker, examined in isolation, reliably distinguishes OTS from health, the same research group responsible for much of the stimulation-testing literature reviewed above developed a series of composite diagnostic scoring systems intended to combine clinical, hormonal, and metabolic parameters into a single, more discriminative instrument [27,30].

The EROS-COMPLETE tool incorporates clinical items, basal and stimulated biochemical measurements, and body composition parameters into a structured scoring system; in a scoping review examining biomarkers and tools potentially diagnostic of OTS, this instrument — together with its simplified and clinical-only variants, EROS-SIMPLIFIED and EROS-CLINICAL — was reported to distinguish all apparently OTS-affected from healthy male athletes in the original study when the three tools were combined sequentially in a decision-tree format, escalating from the purely clinical tool to progressively more biochemically detailed instruments only when an ambiguous result was obtained at an earlier stage [27]. The same

scoping review, however, explicitly cautioned that the claimed 100% diagnostic accuracy of these combined tools lacks a firm methodological foundation given the continued absence of any independent gold-standard diagnostic test against which the instrument's performance could be validated, and it emphasised that the scores require validation in larger samples and, critically, in female athletes, since the foundational cohort comprised exclusively men [27].

Beyond the EROS instruments specifically, the broader scoping review identified a wide diversity of candidate biomarkers and tools across the published literature, spanning basal hormone levels, hormonal responses to the ITT or two-bout exercise protocol, psychological questionnaires (the Profile of Mood States, the Recovery-Stress Questionnaire for Athletes), heart rate variability, electroencephalographic patterns, metabolic alterations assessed via Fourier transform infrared spectroscopy, neurotransmitter measures, immunological and redox parameters, muscular structural changes, and body composition indices, none of which, examined in isolation, achieved the discriminative performance of the composite instruments [27]. This diversity itself is instructive: it reflects the field's underlying recognition, made explicit in the multisystem convergence model discussed in Section 4, that OTS genuinely affects multiple physiological systems simultaneously, such that a composite, multi-domain instrument is likely to be inherently more robust than any single-parameter biomarker, however mechanistically appealing [27].

Several methodological limitations attend this body of work. The scoping review noted that among 39 included publications meeting eligibility criteria, eight were based on a single subject, that endurance athletes were substantially overrepresented relative to resistance or mixed-modality athletes, and that female athletes remained markedly underrepresented across the field as a whole — a pattern consistent across virtually every dataset discussed in this review and one that fundamentally constrains the generalisability of currently available diagnostic thresholds [27]. The review further observed that catecholamine responses appeared to differ qualitatively between endurance and resistance athletes with OTS, with decreased catecholamine levels reported in endurance-sport cohorts but increased levels in resistance-trained weightlifters, a divergence interpreted as potentially reflecting genuinely distinct "sympathetic" and "parasympathetic" phenotypes of OTS corresponding respectively to resistance/power and endurance training backgrounds, an interesting hypothesis that nonetheless requires confirmation through direct, prospective comparison rather than retrospective cross-study synthesis [27].

A further limitation worth stating explicitly concerns the decision-tree structure of the EROS-COMPLETE/SIMPLIFIED/CLINICAL system itself: because the three component tools were derived and validated within the same original cohort rather than through prospective, out-of-sample testing, the reported perfect discrimination may partly reflect overfitting to the idiosyncrasies of that specific dataset rather than a generalisable diagnostic property of the instrument, a concern common to composite scoring systems developed and validated within a single research group's cohort without external, independently collected validation data [27]. Until such external validation is available, ideally in a cohort recruited by an independent research group and including both sexes and multiple training modalities, the EROS composite instruments should be regarded as a promising proof-of-concept rather than as a clinically ready diagnostic tool.

11. Systemic Inflammation, Immune Function, and Overtraining

The cytokine hypothesis introduced in Section 4 warrants closer examination, given its mechanistic centrality to the interaction between HPA-axis function and the broader multisystem phenotype of OTS. Repetitive muscle contraction and joint loading produce

cumulative tissue microtrauma that, under conditions of adequate recovery, resolves through a self-limited local inflammatory response involving IL-1β, IL-6, and TNF-α, followed by subsequent release of anti-inflammatory mediators that restore tissue homeostasis [16,22]. Under conditions of chronic training overload without adequate recovery, this local inflammatory response is proposed to become amplified and to fail to resolve normally, generating cumulative systemic inflammation that interacts with and perpetuates dysfunction across multiple other physiological systems [16,22].

Elevated circulating pro-inflammatory cytokines have been proposed to induce reduced food intake (contributing to the negative energy balance frequently observed in OTS), to interfere directly with insulin-stimulated glucose transporter (GLUT-4) translocation in skeletal muscle (thereby impairing glycogen resynthesis and potentially reinforcing the glycogen depletion hypothesis), and to act directly on the brain to produce the depressive symptomatology, sleep disturbance, and reduced motivation that overlap so substantially with major depressive disorder [16,22]. Mechanistically, IL-1β and TNF-α are understood to be potent activators of the HPA axis at the hypothalamic level, stimulating CRH release and thereby providing a direct pathway by which peripheral inflammatory processes could plausibly drive the central neuroendocrine dysfunction documented in the stimulation-testing literature reviewed in Section 7 — although this proposed causal chain has not, to our knowledge, been directly demonstrated in a single study measuring both cytokine profiles and dynamic HPA-axis function in the same OTS-affected cohort [16,22].

Empirical support for frank clinical inflammation in established OTS is, however, more equivocal than the theoretical mechanism might suggest. As noted in Section 6, the EROS-BASAL study found no significant differences in erythrocyte sedimentation rate, C-reactive protein, or ferritin between OTS-affected athletes, healthy athletes, and sedentary controls, leading the authors to conclude that classical clinical markers of systemic inflammation do not distinguish OTS from health, even though more specific cytokines such as IL-1β, IL-6, and TNF-α were not directly measured in that particular cohort [24]. Elsewhere in the literature, findings regarding specific cytokine elevation in OTS have been described as inconsistent across studies, with some reporting acute increases in IL-6 and IL-2 following exercise but no data on sustained elevation with chronic training, and at least one study specifically examining overtrained cyclists finding no change in IL-6 or TNF-α concentrations, a negative result the original authors speculated might reflect the predominantly concentric nature of cycling muscle contraction producing insufficient microtrauma to trigger a substantial inflammatory cascade relative to eccentric-loading sports such as running [16]. This inconsistency underscores a recurring pattern across the OTS literature: mechanistically plausible hypotheses, well-supported at the level of basic physiology, frequently fail to translate into consistent, reproducible findings when tested empirically in heterogeneous cohorts of naturally occurring OTS.

A closely related line of evidence concerns the immunological consequences of intensified training more broadly, independent of whether frank OTS is present. A systematic review and meta-analysis of lymphocyte and dendritic cell responses to periods of intensified training in young healthy humans and rodents found evidence of transient, exercise-load-dependent alterations in circulating lymphocyte subpopulations and dendritic cell numbers, consistent with a generalised, dose-dependent perturbation of adaptive immune surveillance during periods of heavy training load, though the reviewed studies were heterogeneous in training protocol, species, and outcome measures, limiting the precision with which a specific threshold of training load associated with clinically meaningful immunosuppression could be identified [21]. This body of evidence lends indirect support to the long-observed clinical association

between heavy training load and increased susceptibility to upper respiratory tract infection in athletes, a phenomenon addressed directly by the International Olympic Committee's consensus statement on training load and illness risk, which describes abrupt increases in training load and congested competition scheduling as consistent, if imperfectly quantified, risk factors for illness across multiple sports [7]. Although this consensus statement is not specific to OTS, its underlying immunological rationale — that training load beyond an individual's adaptive capacity compromises immune surveillance via mechanisms overlapping substantially with those proposed under the cytokine and glutamine hypotheses — reinforces the broader multisystem model of training maladaptation that pervades this review.

A related but distinct line of evidence concerns the gut-brain axis and its potential contribution to both the inflammatory and neuropsychiatric dimensions of OTS. Intense and prolonged exercise redistributes blood flow away from the splanchnic circulation toward working muscle and skin, producing intestinal hypoperfusion and increased gut permeability — the so-called "leaky gut" phenomenon — which permits translocation of bacterial lipopolysaccharide into the systemic circulation and thereby amplifies the systemic inflammatory response described above [22,32]. Given that approximately 90% of the body's serotonin is synthesised in the gastrointestinal tract, and that gut microbiota composition can directly influence host tryptophan metabolism and serotonergic tone, chronic exercise-induced intestinal barrier compromise has been proposed as a further, largely unexplored mechanistic link between physical training stress and the central neuropsychiatric symptomatology of OTS, complementing the central serotonergic mechanisms already described under the central fatigue hypothesis [22,32]. This gut-brain axis literature remains, however, considerably more preliminary and mechanistically speculative than the corresponding HPA-axis literature, drawn substantially from animal models and from studies of exercise-associated gastrointestinal symptoms in generally healthy rather than specifically overtrained athletes, and it should be regarded as a promising area for future investigation rather than an established component of OTS pathophysiology.

12. Sex-Specific Considerations and the Female Athlete

A recurring and consequential limitation, already flagged at several points in this review, is that the overwhelming majority of the foundational HPA-axis and hormonal biomarker literature in OTS has been conducted exclusively in male athletes, leaving the applicability of the diagnostic frameworks and proposed thresholds described above to female athletes largely untested [9,24,27]. This omission is not merely a matter of incomplete sampling but carries direct clinical consequence, because several lines of evidence indicate that female athletes may respond differently, both hormonally and behaviourally, to the combination of training, energy, and psychosocial stress that underlies OTS.

Within the TCR literature specifically, cortisol was found to increase significantly across a competitive season in female but not male elite endurance athletes despite similar mean TCR values between sexes, and a reduced TCR predicted poorer race performance specifically in female amateur rowers without a corresponding relationship in male rowers from the same cohort, indicating that identical hormonal patterns may carry different performance implications depending on sex [29]. More broadly, a comprehensive review of stress-related adrenal steroid metabolism under extreme physical and psychological stress in military populations found that women demonstrated distinct patterns of HPA-axis adaptation during prolonged training relative to men, with some evidence of both positive adaptation during training and subsequent maladaptive reproductive and metabolic consequences specific to female personnel that were not mirrored in male cohorts undergoing comparable training loads [33]. This body of work, though derived predominantly from military rather than athletic

populations, underscores that sex is not simply a demographic variable to be adjusted for statistically but appears to represent a genuine determinant of the magnitude, direction, and clinical significance of HPA-axis and gonadal-axis adaptation to chronic physical stress [33].

Beyond the HPA axis narrowly defined, female athletes experiencing chronic energy deficit — whether related to deliberate caloric restriction, disordered eating, or simply inadequate matching of intake to the energy demands of training — are additionally susceptible to the female athlete triad, comprising low energy availability, menstrual dysfunction, and impaired bone mineral density, a syndrome whose endocrine substrate (functional hypothalamic amenorrhoea driven by GnRH pulse suppression) directly parallels, and substantially overlaps with, mechanisms proposed for OTS [6]. Functional hypothalamic amenorrhoea itself remains a diagnosis of exclusion requiring the systematic elimination of organic causes, and it is characterised hormonally by insufficient hypothalamic GnRH pulsatility, consequent reduction in luteinizing hormone and follicle-stimulating hormone, and downstream hypoestrogenism, with prevalence estimates of secondary amenorrhoea reaching as high as 69% in some elite athlete populations compared with only 2-5% in the general population [6]. The considerable overlap between this hormonal cascade and the broader low energy availability framework underlying RED-S, discussed in the following section, illustrates that in female athletes specifically, HPA-axis assessment for suspected OTS cannot meaningfully proceed without simultaneous, careful evaluation of menstrual status, energy availability, and bone health, since disentangling a primary training-overload phenotype from a primary energy-deficiency phenotype may be genuinely difficult, or in some cases may represent a false dichotomy given their shared upstream drivers.

Beyond these mechanistic considerations, the practical consequence of the near-total absence of female-specific research extends to every proposed diagnostic threshold discussed throughout this review: the EROS-HPA axis cutoffs for ITT cortisol and ACTH response, the proposed T:E ratio threshold of 13.7:1, and the composite EROS scoring instruments were all derived exclusively from male cohorts, meaning that a female athlete presenting with suspected OTS today can be assessed using none of these thresholds with any confidence, and clinicians are left, in practice, to apply population norms of uncertain applicability or to rely on clinical judgement and exclusion of RED-S and functional hypothalamic amenorrhoea as the primary diagnostic anchors, rather than on any validated hormonal cutoff specific to OTS itself [6,9,24,27,30].

13. Differential Diagnosis I: Relative Energy Deficiency in Sport

Relative Energy Deficiency in Sport (RED-S), formally redefined in the International Olympic Committee's 2023 consensus statement, describes a condition arising from chronic low energy availability that produces negative effects across physiological, metabolic, and psychological domains, and it represents arguably the single most important differential diagnosis to exclude, or to characterise as a contributing factor, before OTS can be confidently diagnosed [13]. Unlike the original Female Athlete Triad construct from which it evolved, RED-S is now recognised to affect male as well as female athletes, and its endocrine signature — suppressed leptin, reduced triiodothyronine (T3), suppressed insulin-like growth factor-1, and suppressed reproductive hormone concentrations — can occur even in athletes maintaining apparently normal body weight and composition, meaning that the absence of visible weight loss cannot be used to exclude the condition [13].

A systematic review directly comparing RED-S and OTS in endurance athletes concluded that the two syndromes, while producing substantially overlapping clinical presentations — fatigue, illness susceptibility, performance decline, and mood disturbance — arise from fundamentally

distinct primary mechanisms: RED-S from chronic low energy availability and OTS from chronic imbalance between training load and recovery capacity, a distinction with direct and divergent management implications, since treating RED-S as though it were OTS delays essential nutritional rehabilitation, while treating OTS as though it were RED-S fails to address the training-load excess that is actually driving the presentation [13]. This same review identified autonomic function as perhaps the clearest single point of biochemical differentiation currently available: athletes with OTS consistently demonstrate reduced heart rate variability, elevated resting heart rate, and impaired sympathetic-parasympathetic balance, whereas RED-S does not consistently alter autonomic function unless compounded by substantial additional psychological stress or concurrent overreaching, a pattern that — if confirmed in larger, prospectively designed cohorts — could provide a genuinely useful bedside discriminator between the two conditions [13].

Endocrine patterns likewise diverge in ways consistent with their distinct proposed mechanisms: RED-S produces a relatively predictable, reproducible pattern of hormonal suppression driven directly by caloric insufficiency and detectable regardless of body mass, whereas cortisol responses documented in OTS are considerably more variable and context-dependent, ranging across different studies from blunted to exaggerated depending on the timing of measurement relative to training load and recovery status, such that cortisol alone was explicitly judged insufficient, on its own, to reliably distinguish established OTS either from RED-S or from simple training fatigue [13]. Performance trajectories over time offer a further point of distinction that may ultimately prove more clinically useful than any single biomarker: RED-S-related performance decline typically improves progressively once adequate energy availability and appropriate nutritional periodisation are restored, whereas athletes with genuine OTS may remain functionally impaired for months despite objectively adequate nutritional intake, their recovery instead depending on sustained reduction of training load and comprehensive attention to the broader recovery environment [13].

A substantial body of evidence nevertheless supports genuine mechanistic overlap and bidirectional interaction between the two conditions rather than a clean, mutually exclusive dichotomy: chronic underfuelling appears to accelerate the transition from functional to non-functional overreaching and ultimately to established OTS, since inadequate energy availability directly compromises the physiological capacity to recover from a given training load, while the combined presentation of both conditions together has been associated with more severe endocrine suppression, elevated bone stress injury risk, and substantially prolonged return-to-competition timelines relative to either condition occurring in isolation [13]. Given this overlap, the systematic review's authors recommended dual-condition screening — encompassing detailed energy intake assessment, comprehensive hormonal profiling, quantified training load analysis, heart rate variability monitoring, injury history, and psychological screening — as the most defensible current approach for any athlete presenting with persistent, otherwise unexplained fatigue or performance decline, rather than attempting to force a binary diagnostic choice between the two entities on the basis of any single test result [13].

A further point worth emphasising is that low energy availability, the proximate physiological driver of RED-S, has itself been directly shown to activate the HPA axis and to suppress gonadotrophic and thyroid function through mechanisms substantially overlapping with those proposed for OTS, meaning that the two conditions may, in some athletes, represent different entry points into a shared final common endocrine pathway rather than genuinely independent processes [20]. Prospective studies of experimentally induced low energy availability in both female and male participants have documented dose-dependent suppression of luteinizing hormone pulsatility, triiodothyronine, and IGF-1, alongside elevations in cortisol that scale with

the severity and duration of the energy deficit, findings that mirror, at least qualitatively, several of the hormonal abnormalities documented in the OTS literature reviewed in Sections 6 through 9 [20]. This convergence strengthens the argument, made explicitly in Section 18, that careful assessment of energy availability should be considered a mandatory component of any HPA-axis-focused OTS work-up, rather than a separate, optional line of enquiry pursued only when RED-S is specifically suspected on other clinical grounds.

14. Differential Diagnosis II: Major Depressive Disorder and the Psychiatric Dimension

A second, equally consequential area of diagnostic overlap concerns major depressive disorder (MDD) and other primary psychiatric conditions, whose neurobiological substrate shares such striking mechanistic parallels with the proposed pathophysiology of OTS that some authors have explicitly proposed a partially common underlying origin for the two conditions [15,34].

Both OTS and MDD are characterised by dysregulation of HPA-axis function, though the direction of this dysregulation in MDD has itself been described as heterogeneous across the depression literature, encompassing patterns of both hypercortisolism with impaired negative feedback and, in some cohorts, blunted cortisol responses reminiscent of those documented in OTS, alongside altered glucocorticoid and mineralocorticoid receptor sensitivity [34,35]. A comprehensive review of the interaction between the HPA axis and the gut-brain axis in depression described how chronic HPA-axis hyperactivation can produce hippocampal atrophy, reduced neurogenesis, and impaired synaptic plasticity via reduced expression of brain-derived neurotrophic factor (BDNF), alongside amplification of pro-inflammatory cytokine signalling — IL-1, IL-6, and TNF-α — that in turn can cross the blood-brain barrier to worsen central neuroinflammation, establishing a self-perpetuating cycle between peripheral inflammation, HPA-axis dysfunction, and depressive symptomatology that closely mirrors the mechanistic model proposed for OTS in Sections 4 and 11 [34].

Beyond the HPA axis narrowly defined, both OTS and depression are proposed to involve dysregulated central serotonergic and dopaminergic signalling, with reduced availability of tryptophan for serotonin synthesis in states of systemic inflammation (because tryptophan is diverted toward the kynurenine pathway under the influence of indoleamine 2,3-dioxygenase, itself upregulated by inflammatory cytokines), a mechanism directly paralleling — and potentially converging with — the central fatigue hypothesis described in Section 4 [16,34]. At a neurological and behavioural level, a narrative review specifically addressing the neurological dimension of OTS in competitive dancers described HPA-axis dysregulation, monoaminergic neurotransmitter depletion (particularly of serotonin and dopamine), neuroinflammation, and disrupted sleep architecture with suppressed BDNF expression as jointly producing a clinical picture of anhedonia, impaired motor memory consolidation, reduced motivation, and cognitive "fog" that is, by the reviewing authors' own explicit acknowledgement, difficult to distinguish reliably from a primary depressive episode on clinical grounds alone [17].

A literature review specifically addressing the mental health dimension of OTS proposed that the most clinically useful discriminator between OTS and primary MDD, in the absence of a validated biological test capable of reliably separating the two, is the domain of functional impairment: impairment in OTS is typically restricted to sport-specific performance and improves, at least to some degree, with rest, whereas depression characteristically produces pervasive impairment across social, occupational, and personal domains and may in fact worsen, rather than improve, with enforced inactivity, since exercise itself may serve as an important coping mechanism for some individuals [15]. This same review candidly acknowledged, however, that this distinction is far from absolute in clinical practice, particularly in athletes with pre-existing depressive vulnerability, in whom OTS may plausibly

unmask or directly precipitate a genuine primary depressive episode requiring independent psychiatric treatment in its own right, meaning that careful, individualised psychiatric assessment — rather than reliance on any biochemical algorithm — remains indispensable in the evaluation of any athlete presenting with the combination of fatigue, underperformance, and mood disturbance [15]. This literature review, focused specifically on the intersection of overtraining and mental health outcomes in athletic populations, provides the primary evidentiary basis for the psychiatric symptom-domain framework discussed further in Section 15, and its explicit emphasis on functional-impairment pattern, rather than any single biochemical marker, as the most practically useful bedside discriminator is a recurring theme that this review considers among its most clinically actionable conclusions [15].

Table 1 summarises the principal features distinguishing, and connecting, the three overlapping conditions discussed in Sections 13 and 14 — OTS, RED-S, and major depressive disorder — alongside the primary HPA-axis-relevant hormonal patterns reported for each across the sources reviewed in this article.

Table 1. Comparative features of overtraining syndrome, relative energy deficiency in sport, and major depressive disorder as reported across the reviewed sources.

Feature	**Overtraining syndrome**	**Relative energy deficiency in sport**	**Major depressive disorder**
Primary proposed driver	Chronic training-load/recovery imbalance	Chronic low energy availability	Multifactorial neurobiological/psychosocial
Basal cortisol/ACTH	Generally unremarkable; inconsistent across studies	Not the primary reported hormonal signature	Heterogeneous: hypercortisolism or blunted response reported
Stimulated HPA response	Blunted late cortisol, ACTH, GH response reported in ITT/two-bout protocols	Not systematically characterised in reviewed sources	Impaired negative feedback; heterogeneous patterns reported
Key hormonal signature	Reduced testosterone-to-estradiol ratio; blunted GH/prolactin stimulation	Suppressed leptin, T3, IGF-1, reproductive hormones	Altered glucocorticoid/mineralocorticoid receptor sensitivity
Autonomic function	Reduced heart rate variability, elevated resting heart rate	Not consistently altered unless compounded by additional stress	Not characterised in reviewed OTS-focused sources
Recovery pattern	May persist despite adequate nutrition; requires load reduction	Improves with restored energy availability	Requires independent psychiatric management; may worsen with rest

Functional impairment domain	Sport-specific	Sport-specific, with systemic metabolic/bone effects	Pervasive across social, occupational, personal domains

Sources: [1,9,13,15,24,29,34].

15. Neurological and Psychiatric Manifestations Beyond the HPA Axis

While the preceding sections have concentrated on hormonal and endocrine aspects of OTS, the syndrome's neurological and psychiatric dimensions merit direct attention in their own right, both because they represent a substantial component of the clinical burden reported by affected athletes and because several of the mechanisms proposed to underlie them connect directly back to HPA-axis dysfunction.

A literature review specifically addressing overtraining syndrome and mental health in athletes described three overlapping symptom domains characterising the psychiatric presentation of OTS: an affective and anxiety-related domain encompassing depressed mood, irritability, emotional lability, anhedonia, and apathy, frequently emerging before objective performance decrements become clinically apparent; a neurocognitive and somatic domain encompassing sleep disturbance, impaired concentration and working memory, slowed processing speed, and persistent appetite and gastrointestinal disturbance; and an athlete burnout domain characterised by emotional exhaustion, depersonalisation from the sport, and a reduced sense of personal accomplishment [15]. This same review noted that the Profile of Mood States "iceberg profile" — characterised in psychologically healthy athletes by vigour scores exceeding all negative mood subscales — progressively inverts as athletes transition from health through non-functional overreaching to established OTS, with rising fatigue and depression scores and falling vigour providing a trackable, non-invasive indicator that may precede objective performance decline by days to weeks and could therefore, in principle, serve a useful early-warning function in routine athlete monitoring [15].

A separate narrative review addressing the specific neurological demands of competitive dance proposed that the unusually dense cognitive-motor requirements of this discipline — combining extreme physical exertion with precise proprioceptive, rhythmic, and artistic demands — may render dancers particularly vulnerable to a specific constellation of OTS-related neurological symptoms including impaired proprioceptive integration, disrupted execution of highly automatised motor programmes ("motor engrams"), and impaired musical-rhythmic timing, each attributed mechanistically to a combination of dopaminergic depletion, cerebellar and basal ganglia dysfunction, and sleep-dependent impairment of motor memory consolidation [17]. This same review proposed heart rate variability monitoring, together with validated psychometric instruments such as the RESTQ-Sport questionnaire and structured neurocognitive screening, as practical tools for the early identification of at-risk dancers, while explicitly acknowledging the largely extrapolated, rather than dance-specific, nature of much of the underlying mechanistic evidence, given the near-total absence of dedicated neurological research in this particular athletic population [17].

At the level of basic neuroscience, a controlled animal study examining the effects of a structured six-week overtraining protocol in mice found that overtrained animals exhibited anxiety-like behavioural changes on open-field testing — fewer entries into and reduced time spent in the arena's centre zone — together with reduced hippocampal expression of the tight-junction protein zonula occludens-1 (ZO-1) and elevated hippocampal TNF-α, in contrast to animals subjected to a moderate, non-excessive exercise protocol, which instead showed increased expression of the tight-junction protein claudin-5, interpreted by the study's authors

as reflecting a protective, adaptive blood-brain barrier response specific to moderate, appropriately dosed training [31]. While this animal model cannot establish equivalent mechanisms in human athletes, and the study's authors themselves noted a substantially reduced final sample size (four mice in the overtraining group, following exclusion of six animals that developed training-related tail or hindlimb injuries) that limits the statistical power and generalisability of the findings, it offers a biologically plausible mechanistic bridge — neuroinflammation-associated blood-brain barrier compromise — potentially linking the peripheral cytokine elevation proposed under the cytokine hypothesis (Section 11) directly to the central anxiety and mood symptomatology documented clinically in overtrained human athletes, and it represents one of the only sources reviewed here to attempt a direct, controlled, mechanistic test of the neuroinflammation-behaviour link in an overtraining model rather than relying solely on cross-sectional or correlational human data [31].

Taken together, the psychiatric and neurological literature reviewed in this section and in Section 14 converges on a picture in which mood disturbance is not a peripheral or secondary feature of OTS but an integral, and in some formulations perhaps even a defining, component of the syndrome — a conclusion with direct implications for clinical practice, since it argues strongly against approaching suspected OTS as a purely endocrine or musculoskeletal problem and in favour of routinely incorporating structured psychological assessment into any diagnostic work-up, regardless of whether formal psychiatric symptoms are volunteered spontaneously by the athlete.

16. Prevention, Monitoring, and Early Detection

Given the persistent absence of a single validated diagnostic biomarker for OTS, the practical clinical emphasis across the reviewed literature has shifted decisively toward prevention and early, longitudinal, multi-domain monitoring rather than reliance on any single confirmatory test applied at a single point in time.

Systematic psychological monitoring using validated instruments — the Profile of Mood States, the Recovery-Stress Questionnaire for Athletes, and brief training-distress scales — is consistently identified as among the most practically useful available tools, on the basis that affective changes have been shown to precede objective performance decrements by days to weeks, offering a genuine window for intervention before the more severe, potentially prolonged features of established OTS become manifest [15]. A specific practical example cited in the literature describes how systematic POMS-guided training-load adjustment among collegiate swimmers was associated with a reduction in reported burnout incidence from 10% to zero across a competitive season, illustrating the potential real-world value of routine mood monitoring integrated directly into training decision-making, although this remains a single observational example rather than a controlled trial [15].

At the physiological level, heart rate variability has emerged across multiple independent sources as a promising, non-invasive, and readily repeatable candidate marker of autonomic dysregulation in both non-functional overreaching and established OTS, distinct from, though potentially complementary to, the hormonal stimulation-testing approaches discussed in Section 7 [13,15,17]. Daily monitoring of session ratings of perceived exertion (RPE) has similarly been proposed as a simple, low-cost frontline signal, on the basis that an overtrained athlete characteristically reports an elevated RPE for a given absolute external training load, potentially flagging emerging maladaptation before more invasive or expensive biochemical testing becomes necessary or clinically indicated [15].

At a broader structural level, the International Olympic Committee's consensus statement on training load and illness risk, though addressing load management and illness susceptibility

rather than OTS specifically, offers directly relevant guidance: it describes an evidence-informed, though sport-specific and imperfectly quantified, relationship between abrupt increases in training load, insufficient recovery, congested competition calendars, and elevated illness and injury risk, and it provides structured, practical recommendations spanning training-load periodisation, monitoring of both external load (volume, intensity) and internal load (subjective wellness, heart rate response), psychological stress management, and behavioural and nutritional strategies to support robust immune function during periods of heavy training [7]. Although this consensus statement is framed principally around infectious illness risk rather than OTS as such, its underlying emphasis on individualised, incremental load progression and on the integration of external and internal load monitoring rather than reliance on either measure alone is directly applicable to, and consistent with, the broader preventive philosophy that pervades the OTS-specific literature reviewed throughout this article.

A further preventive consideration, less frequently emphasised in the OTS-specific literature but directly implied by the mechanistic overlap discussed in Section 13, concerns systematic monitoring of energy availability alongside training-load and psychological metrics, since the evidence reviewed there indicates that inadequate energy intake can independently accelerate the transition from functional overreaching to established OTS regardless of training-load management [13,20]. A genuinely comprehensive monitoring framework, consistent with the multisystem model of OTS pathophysiology developed throughout this review, would therefore integrate at minimum four parallel data streams — training load (external and internal), psychological state, autonomic function, and energy availability/nutritional status — rather than treating any single stream as sufficient on its own, an approach that mirrors, at the level of practical monitoring, the same multi-domain philosophy underlying the composite EROS diagnostic instruments discussed in Section 10.

17. Treatment Approaches and Return-to-Training Considerations

Once OTS is suspected or diagnosed, the management approaches described across the reviewed literature are notably consistent despite the considerable underlying diagnostic uncertainty discussed in preceding sections, and they converge on structured reduction or temporary cessation of training as the primary intervention, undertaken within a multidisciplinary clinical framework.

The ECSS/ACSM consensus statement and subsequent reviews describe no specific pharmacological treatment validated for OTS itself, and recommend instead a graduated return-to-training protocol — beginning with complete rest or substantially reduced training volume, followed by gradual reintroduction of low-intensity activity, and only later progressive restoration of prior training intensity and volume, guided throughout by objective markers of recovery (mood questionnaire scores, heart rate variability, and where available, biochemical indicators) rather than by a fixed, predetermined timeline [1,15]. This approach reflects the practical reality that recovery from established OTS may extend across weeks to months, or in some reported cases considerably longer, and that premature return to full training load risks precipitating relapse, consistent with the high recurrence rates noted in Section 3 [1,15,17].

A literature review on mental health in athletes specifically recommended that complete rest not be regarded as a uniformly appropriate intervention, since some athletes for whom exercise constitutes a primary coping mechanism may, paradoxically, experience worsening of mood and anxiety symptoms during enforced complete inactivity; in such cases, relative rest incorporating low-intensity active recovery, combined with structured psychological support, may represent a more clinically appropriate strategy than total cessation of physical activity [15]. Where psychiatric comorbidity meeting formal diagnostic criteria for MDD or an anxiety

disorder is identified — a possibility that, as discussed in Section 14, must be actively considered given the substantial symptomatic overlap — independent psychiatric treatment following standard clinical guidelines is recommended, with the explicit caveat that selective serotonin reuptake inhibitors and other serotonergic antidepressants may themselves reduce exercise performance and should therefore be prescribed only following careful, shared decision-making between the athlete, treating physician, and psychiatric specialist [15,19].

Where RED-S is identified as a contributing or primary factor, as discussed in Section 13, nutritional rehabilitation — specifically, restoration of adequate energy availability appropriately matched to training demands — represents an essential and non-negotiable component of management, distinct from, though potentially concurrent with, any indicated reduction in training load [13]. Given the considerable evidence, reviewed throughout Sections 6 through 9, that OTS involves dysfunction across multiple interacting hormonal axes rather than isolated HPA-axis pathology alone, the overall weight of the reviewed literature supports a genuinely multidisciplinary management approach integrating sports medicine physicians, endocrinological expertise where indicated, sports psychiatry or psychology, and dietetic input, rather than management directed at any single presumed causal pathway.

An additional practical management consideration, implicit throughout the literature reviewed in Sections 7 through 9 but rarely stated explicitly, concerns the monitoring of recovery itself once training reduction has been instituted: because basal hormone measurements have consistently failed to discriminate OTS from health in the diagnostic setting, it seems reasonable to expect that they would likewise be of limited value in confirming resolution of the syndrome during the recovery phase, and that serial reassessment using the same composite, ratio-based, or stimulation-testing approaches shown to have greater discriminative value at diagnosis would similarly be more informative in tracking recovery, although direct longitudinal data specifically addressing this question during the recovery phase remain comparatively sparse within the sources reviewed here.

18. Discussion

Read as a whole, the evidence assembled in this review supports several convergent conclusions while simultaneously revealing the persistent limitations that continue to constrain both mechanistic understanding and clinical practice in this field.

First, HPA-axis dysfunction in OTS is genuinely documented but is neither uniform nor straightforwardly interpretable as a simple linear progression from hyperactivation to exhaustion. Basal cortisol and ACTH measurements, despite their intuitive appeal and historical primacy in the field, have been shown with remarkable consistency across independent datasets to fail as discriminators between OTS-affected and healthy athletes [9,10,24]. Dynamic stimulation testing, by contrast, more reliably exposes a pattern of blunted late hormonal responsiveness — to insulin-induced hypoglycaemia, and in a somewhat different paradigm, to a repeated maximal exercise challenge — that is mechanistically plausible as an explanation for the reduced time-to-fatigue characteristic of established OTS, but this literature remains concentrated within a small number of research groups, predominantly examining small, male-only cohorts of combined endurance/resistance athletes, and it has not been prospectively validated as a predictive tool in athletes at risk rather than retrospectively characterised in those already diagnosed [9,12,24].

Second, the evidence repeatedly demonstrates that HPA-axis findings cannot be interpreted in isolation from the broader hormonal and metabolic context, and that composite or ratio-based measures — the testosterone-to-estradiol ratio in particular, and to a lesser and more contested extent the testosterone-to-cortisol ratio — appear to carry more discriminative value than any

single hormone considered alone [24,29,30]. This finding reconciles much of the apparent inconsistency across the broader literature: studies examining single hormones in isolation, at a single timepoint, without reference to an individual athlete's own baseline, are almost certain to yield conflicting or null results, whereas studies examining hormonal ratios, stimulated rather than basal responses, or serial within-athlete trajectories consistently perform better, a pattern entirely consistent with the broader recognition, discussed in Section 4, that OTS reflects the convergent failure of multiple interacting regulatory systems rather than isolated dysfunction of any single axis.

Third, the substantial mechanistic and symptomatic overlap between OTS and both RED-S and major depressive disorder is not merely a diagnostic inconvenience to be overcome through more precise biomarker development, but appears to reflect genuine, biologically meaningful convergence of shared upstream pathways — chronic energy insufficiency, HPA-axis dysregulation, neuroinflammation, and monoaminergic disturbance — that manifest with overlapping downstream phenotypes across what may in reality be a spectrum of related conditions rather than three cleanly separable diagnostic categories [13,15,34]. This observation carries a direct clinical implication: rather than searching for ever more specific biomarkers intended to separate OTS definitively from its principal differential diagnoses, the field may be better served by accepting that careful, systematic, multi-domain clinical characterisation — encompassing training history, energy availability, menstrual and reproductive function, psychiatric symptomatology, and autonomic function alongside whatever hormonal testing is undertaken — will likely remain indispensable regardless of further biomarker refinement.

Fourth, the near-universal underrepresentation of female athletes across the foundational hormonal biomarker literature represents perhaps the single most consequential limitation identified in this review, given the direct evidence that sex modifies both the hormonal response pattern itself and its clinical and performance significance [27,29,33]. Diagnostic thresholds and composite scoring instruments derived exclusively from male cohorts cannot be assumed, without direct testing, to apply to female athletes, particularly given the well-documented interaction between energy availability, reproductive hormone status, and HPA-axis function that is specific to, or at minimum substantially more pronounced in, women.

Fifth, comparing the relative strength of evidence across the various components of the HPA-axis hypothesis reviewed in this article reveals a clear gradient that has not, in our reading, been made sufficiently explicit in prior narrative treatments of this topic. At the strongest end of this gradient sits the repeated, cross-study demonstration that basal cortisol and ACTH fail to discriminate OTS from health — a finding that has now been replicated across a systematic review spanning up to 38 studies and an independent cross-sectional cohort study, and which can reasonably be regarded as settled within the limits of current evidence [9,10,24]. At an intermediate level of confidence sits the ITT-based demonstration of blunted late cortisol, ACTH, GH, and prolactin responses, which, while internally consistent across the EROS studies and broadly compatible with earlier two-bout protocol findings, derives predominantly from a single research group and a modest total sample size, and therefore warrants a more provisional interpretation pending independent replication [9,12,30,31]. At the weakest end of this gradient sit the composite diagnostic instruments themselves, which, despite their intuitive and practically attractive multi-domain design, report a level of diagnostic accuracy (100% discrimination in the originating cohort) that exceeds what would ordinarily be expected of any biomedical instrument validated only within its own derivation sample, and which must accordingly be regarded as unproven pending external validation [27]. Explicitly ranking the evidence in this way — rather than treating all findings reviewed in Sections 6 through 10 as possessing comparable evidentiary weight — is, in our view, essential for clinicians and

researchers attempting to translate this literature into either clinical practice or future study design.

Finally, the field's continued reliance on retrospective, cross-sectional, small-sample studies — a constraint imposed substantially by the ethical impossibility of experimentally inducing genuine OTS in human volunteers — means that causal inference throughout this literature must be regarded with considerable caution. Correlational findings linking hormonal patterns to mood, body composition, or performance cannot, on their own, establish the direction of causality, and the field's reliance on naturally occurring cases, while necessary, introduces additional heterogeneity related to the diverse and uncontrolled combination of training, nutritional, sleep, and psychosocial stressors that any individual affected athlete happens to have experienced prior to diagnosis.

19. Limitations of the Evidence Base

Several limitations qualify the conclusions drawn throughout this review and warrant explicit acknowledgement. First, this is a narrative rather than a systematic review: source selection, thematic grouping, and interpretive emphasis were not governed by a registered protocol, no formal risk-of-bias assessment was undertaken, and no quantitative synthesis of effect sizes was attempted, meaning that the review's conclusions reflect a reasoned synthesis of the available literature rather than a formally graded body of evidence. Second, and as emphasised repeatedly throughout, the foundational hormonal and stimulation-testing literature on OTS derives overwhelmingly from small, single-sex (predominantly male), single-research-group cohorts, frequently comprising fewer than 20 participants per study arm, which substantially constrains statistical power and external generalisability [9,10,24,30]. Third, the absence of a validated gold-standard diagnostic test for OTS means that every study included in this review necessarily relied on clinical, exclusion-based case definitions that varied, at least in detail, across research groups and time periods, introducing an unquantifiable degree of case-definition heterogeneity that likely contributes to the inconsistency of findings documented across Sections 6 through 10. Fourth, the ethical impossibility of experimentally inducing true OTS in human research participants means that virtually all human evidence is either cross-sectional, comparing naturally occurring cases against matched controls, or derives from shorter-duration, non-functional overreaching models that may not fully replicate the physiology of established, chronic OTS [1,12,27]. Fifth, animal model data, while offering valuable mechanistic insight unattainable in human studies — as in the neuroinflammation and blood-brain barrier findings discussed in Section 15 — cannot be directly extrapolated to human athletes without independent confirmation, and the specific animal study reviewed here was itself constrained by a substantially reduced final sample size following unplanned attrition [31]. Sixth, this review's coverage of the gut-brain axis, neurosteroid pathways, and adipokine signalling in relation to OTS, while included for completeness given their mechanistic relevance to HPA-axis function, rests on a comparatively thinner and more preliminary evidence base than the corresponding cortisol- and gonadotrophin-focused literature, and conclusions in these areas should accordingly be regarded as considerably more provisional. Seventh, the practical and safety-related constraints on stimulation testing discussed in Section 7.1 mean that even the most methodologically robust findings in this domain are unlikely to be directly translatable into routine, large-scale clinical screening, a consideration that should temper enthusiasm for the ITT specifically as a future frontline diagnostic tool regardless of further replication of its research findings. Finally, because this review was restricted to the specific set of sources available for synthesis, it cannot claim to represent an exhaustive account of the entire published OTS literature, and areas of active research not represented within the source material available for this review — including, potentially, emerging multi-omics and biomarker-panel

approaches described in the broader sports-science literature — may bear directly on the questions addressed here without having been considered.

20. Conclusions

Hypothalamic-pituitary-adrenal axis dysfunction constitutes a genuine, mechanistically coherent, and partially characterised component of overtraining syndrome, but it does not, on the basis of the evidence reviewed here, function as a sufficient or independently specific diagnostic criterion. Basal cortisol and ACTH measurement, despite decades of investigation, offer little discriminative value, whereas dynamic stimulation testing — particularly late-phase hormonal responses to insulin-induced hypoglycaemia or to repeated maximal exercise challenge — more consistently, though still imperfectly, identifies a pattern of blunted pituitary responsiveness in affected athletes relative to healthy, trained comparators. This pattern appears to reflect a broader, non-axis-specific reduction in hypothalamic-pituitary reactivity rather than isolated corticotropic dysfunction, evidenced by the strong intercorrelation observed between cortisol, growth hormone, and prolactin responses to identical stimulation protocols. Composite hormonal ratios, particularly the testosterone-to-estradiol ratio, and multi-domain composite diagnostic instruments combining clinical, hormonal, and metabolic parameters, show greater promise than any single biomarker, though the evidence base supporting these instruments remains concentrated within a small number of predominantly male cohorts and awaits independent, sex-inclusive validation.

The persistent diagnostic uncertainty that characterises this field arises not from any single methodological failing but from the genuinely multisystem nature of OTS, which engages HPA-axis, gonadal, somatotropic, autonomic, immune, gut-brain, and central neurotransmitter pathways simultaneously and which overlaps substantially, at both the mechanistic and symptomatic level, with relative energy deficiency in sport and major depressive disorder. Meaningful further progress in this field is likely to depend less on the identification of a single novel biomarker and more on the standardisation of stimulation-testing protocols across research groups, the deliberate inclusion of female athletes and diverse training modalities in future cohorts, and the continued development and prospective, external validation of multi-domain composite instruments that explicitly integrate hormonal, autonomic, psychological, and nutritional data within a framework anchored to each individual athlete's own longitudinal baseline rather than to population-derived thresholds of uncertain applicability.

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