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Exercise-induced Biomarkers in Methamphetamine Addiction: Molecular Mechanisms and Clinical Implications*

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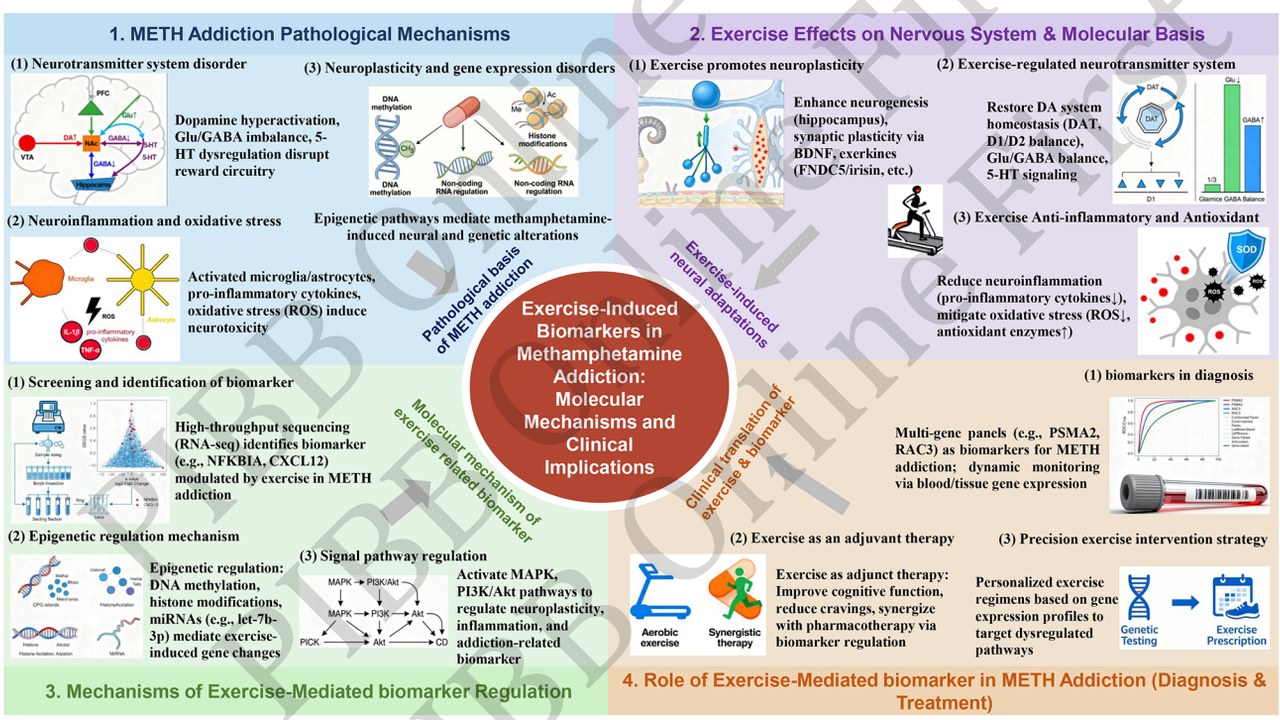
Graphical abstract



Abstract Methamphetamine (METH) addiction is a severe and increasingly prevalent neuropsychiatric disorder for which current diagnostic and therapeutic approaches remain limited and predominantly symptom-oriented. Exercise, as a safe, accessible and cost-effective non-pharmacological intervention, has emerged as a promising strategy to ameliorate METH-induced neurotoxicity and addiction-related behaviors. Growing evidence indicates that these benefits are closely linked to the regulation of

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exercise-induced biomarkers, defined as molecular indicators whose expression or activity is dynamically altered during or after physical activity. This review focuses on the core regulatory role of exercise-induced biomarkers in METH addiction and systematically summarizes their involvement in key neurobiological pathways, outlining molecular pathological mechanisms such as dysregulation of dopamine, glutamate and GABA neurotransmitter systems, neuroinflammation and oxidative stress, and epigenetic remodeling, and emphasizing how these processes converge on changes in candidate biomarkers in the brain and periphery. On this basis, the review describes how exercise modulates neural plasticity, neurotransmitter systems, inflammation and oxidative stress through biomarkers such as brain-derived neurotrophic factor (BDNF), exerkines, inflammatory cytokines, metabolites and non-coding RNAs, with particular attention to neurotrophic and immune-related markers, microRNAs and other epigenetic regulators that can reverse METH-induced synaptic and structural abnormalities and promote recovery of cognitive and emotional functions. Advances in high-throughput omics technologies, including transcriptomics, metabolomics and multi-omics integration, are summarized to illustrate the screening and identification of key exercise-responsive biomarkers. Studies in METH-addicted animal models have revealed differentially expressed genes, signaling pathways (*e.g.*, PI3K-Akt, mTOR, Wnt) and core nodes such as NFKBIA and CXCL12 that may mediate the protective effects of exercise. The review further discusses the potential of exercise-mediated biomarkers as objective indicators for diagnosis, dynamic monitoring of therapeutic efficacy and patient stratification. Multi-gene diagnostic models based on peripheral samples (*e.g.*, hair follicles, blood) demonstrate how biomarker panels can distinguish non-recovered, almost-recovered and healthy individuals, providing a molecular basis for staging METH use disorder and evaluating the impact of exercise interventions. The temporal dynamics of biomarker changes before and after exercise are highlighted, underscoring the value of longitudinal monitoring of factors such as BDNF, immune-related genes and circulating microRNAs to capture treatment-relevant windows of plasticity. In addition, the underlying molecular basis of exercise as an adjunct therapy and gene-targeted exercise strategies that leverage individual biomarker and gene expression profiles to optimize exercise prescriptions are summarized. Current conceptual and technical challenges are outlined, including heterogeneity of biomarker responses, individual variability, assay sensitivity and specificity, and gaps between preclinical findings and clinical application, together with future directions for integrating exercise with multi-omics, artificial intelligence-assisted biomarker discovery and, prospectively, gene-editing-based interventions. Particular emphasis is placed on the need to standardize exercise protocols, incorporate stage-specific and sex-sensitive designs, and combine exercise with pharmacotherapy and psychosocial rehabilitation in real-world clinical settings across diverse healthcare systems. Overall, this review aims to provide a comprehensive and integrated mechanistic framework and updated theoretical support for the application of exercise-mediated biomarkers in the diagnosis, therapeutic effect monitoring and personalized intervention of METH addiction, and to offer new and clinically relevant insights into the development of precision medicine strategies for substance use disorders.

Key words Methamphetamine addiction, exercise, biomarkers, diagnosis, treatment
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Methamphetamine (METH) addiction has emerged as a critical public health challenge worldwide, characterized by its highly addictive nature and profound impact on the central nervous system (CNS). The global prevalence of METH abuse has escalated significantly over recent decades, resulting in widespread neuropsychiatric, cardiovascular, and social consequences. Epidemiological data indicate that METH addiction affects diverse populations, with increasing incidence among both males and females, and imposes severe burdens on healthcare systems and communities[1-2]. The drug's potent psychostimulant properties disrupt normal neurotransmitter dynamics, particularly dopamine, glutamate, norepinephrine, and serotonin systems, leading to complex neurochemical alterations that underpin compulsive drug-seeking and relapse behaviors[23]. Moreover, METH contamination extends beyond human health, as environmental studies have demonstrated its presence in aquatic ecosystems, inducing addiction-like behaviors in wildlife, thereby reflecting the extensive societal and ecological ramifications of this substance[4].

Despite the critical need, effective pharmacological treatments for METH addiction remain elusive. Currently, psychotherapy constitutes the primary treatment modality, yet it is often insufficient in preventing relapse and managing withdrawal symptoms[5]. Immunopharmacotherapies, including vaccine development and monoclonal antibody strategies targeting METH, have shown promise in preclinical and early clinical studies; however, their translation into effective clinical interventions requires further validation of safety and efficacy[56]. Additionally, conventional pharmacotherapies such as naltrexone have demonstrated potential in attenuating METH-induced behavioral sensitization and reward-related memory, indicating a role for opioid receptor antagonists in modulating addiction-related neural circuits[7]. Nevertheless, the high relapse rates and the complexity of METH's neurotoxic effects necessitate innovative approaches that address the multifaceted nature of addiction.

In this context, exercise has emerged as a promising non-pharmacological intervention for

neuropsychiatric disorders, including substance use disorders. Regular physical activity has been shown to ameliorate cognitive deficits, improve mood, and enhance neuroplasticity, thereby offering potential therapeutic benefits in addiction rehabilitation[8]. Experimental evidence from animal models demonstrates that moderate running exercise can accelerate recovery from methamphetamine (METH) addiction, as evidenced by shortened conditioned place preference (CPP) durations and modulation of gene expression profiles in the hippocampus, a brain region critical for learning and memory[8]. Notably, the regulatory effect of exercise on addiction is closely associated with the dynamic changes in exercise-induced biomarkers. Exercise-induced biomarkers refer to molecular biomarkers whose expression levels in the organism undergo alterations during or after exercise, encompassing key molecules such as neurotrophic factors, inflammatory factors, metabolites, and neurotransmitters[9-10]. These biomarkers not only objectively reflect the physiological regulatory effects of exercise on the nervous system, immune system, and metabolic system but also provide crucial clues for deciphering the molecular mechanisms underlying exercise-based intervention in addiction[10]. For instance, exercise-induced upregulation of brain-derived neurotrophic factor (BDNF) has been demonstrated to regulate neuroplasticity as well as emotional and cognitive functions, exhibiting substantial potential in the intervention of neuropsychiatric disorders[10]. In contrast, exercise-mediated regulation of inflammatory factors can alleviate METH-induced neuroinflammation and mitigate neurotoxic damage[9, 11]. Additionally, exercise-induced modulation of immune response-related genes and synaptic plasticity pathways suggests that physical activity may exert neuroprotective effects and facilitate neurobiological recovery in addicted individuals. These findings underscore the potential of exercise as a complementary strategy to conventional treatments, particularly given its accessibility and minimal side effects.

At the molecular level, addiction is increasingly recognized as a disorder involving dysregulation of gene expression and epigenetic modifications that alter neural circuitry and behavior. Markers such as microRNAs (miRNAs), DNA methylation patterns, histone modifications, and non-coding RNAs have been implicated in the modulation of synaptic plasticity and addiction-related memory formation[2, 12]. For instance, specific miRNAs, including hsa-miR-592 and hsa-miR-9-3p, have been identified as potential peripheral biomarkers for METH addiction, with diagnostic capabilities demonstrated through plasma profiling[13]. Epigenetic alterations in genes related to neurotransmitter systems and reward pathways contribute to the persistence of addictive behaviors and represent targets for therapeutic intervention[2, 14]. Furthermore, sex-specific metabolic and molecular signatures have been observed in METH addicts, indicating the necessity for personalized diagnostic and treatment approaches that consider biological sex differences[15-16].

Although existing studies have confirmed that exercise can ameliorate METH addiction-related behaviors by regulating gene expression, neurotransmitter systems, and epigenetic modifications[9, 17], and research on exercise-induced biomarkers has provided preliminary clues for understanding this process, there remain significant limitations in current research. First, most studies focus on the macroscopic effects of exercise on addictive behaviors or investigate changes in biomarkers in isolation. There is a lack of systematic analysis of the synergistic mechanisms between exercise-induced biomarkers, addiction-related genes, and epigenetic regulation, and the specific role of key biomarkers in mediating exercise-induced remodeling of addiction-related neural circuits has not been clarified[9, 18]. Second, the differential regulatory effects of different exercise modalities, intensities, and durations on biomarkers have not been systematically elucidated, making it difficult to develop precise exercise intervention protocols[18-19]. Furthermore, previous studies are mostly based on animal models, and clinical translation studies targeting human METH addicts are relatively scarce. Insufficient attention has been paid to the impacts of individual difference factors such as gender, age, and psychological status on the exercise-biomarker-addiction interaction[20-21], which limits the clinical application of exercise intervention strategies. Additionally, existing biomarker research mostly focuses on single molecules or pathways, lacking integrative analysis of biomarker networks at the multi-omics levels (genomics, transcriptomics, metabolomics)[13, 22], which hinders the comprehensive elucidation of the molecular regulatory networks underlying exercise interventions.

The identification and functional characterization of addiction-related biomarker provide critical insights into the pathophysiology of METH addiction and offer avenues for developing novel diagnostic tools and targeted therapies. Peripheral blood lymphocytes (PBLs) have been proposed as a feasible source for detecting gene expression changes associated with addiction, reflecting central nervous system alterations[23]. Key genes involved in

dopaminergic signaling, opioid receptors, and synaptic function have been identified as potential biomarkers for addiction stages, including abstinence and relapse, highlighting their clinical relevance[23]. Advances in high-throughput sequencing and bioinformatics facilitate the comprehensive profiling of gene expression in accessible tissues, such as whisker follicles and blood, correlating with brain tissue changes and addiction phenotypes[24]. These molecular insights pave the way for integrating biomarker-based diagnostics with behavioral and pharmacological interventions.

Given the limitations of traditional diagnostic and therapeutic approaches in METH addiction, there is an urgent need to explore non-pharmacological intervention strategies such as exercise. This study aims to address the research gap between non-pharmacological interventions and the molecular mechanisms of addiction by systematically elucidating the molecular mechanisms underlying exercise-mediated gene regulation of METH addiction-related biomarkers. It specifically focuses on dissecting the interactions between exercise-induced biomarkers and neurotransmitter systems, neuroinflammation, neuroplasticity, as well as epigenetic modifications. Meanwhile, this study intends to explore and evaluate the application potential of these biomarkers in the diagnosis, therapeutic effect monitoring, and personalized treatment of METH addiction, thereby providing a reference for relevant research and clinical practice on exercise intervention for METH addiction.

1 Molecular pathological mechanisms of methamphetamine (METH) addiction

1.1 Alterations in neurotransmitter systems

METH addiction profoundly disrupts multiple neurotransmitter systems, with the dopamine system being central to its addictive properties due to its critical role in the brain's reward circuitry. The brain reward circuitry targeted by METH primarily centers on the mesolimbic dopamine pathway, characterized by projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), and further engages interconnected brain regions including the prefrontal cortex (PFC), hippocampus, and amygdala, which collectively mediate reward processing, drug-associated memory, and cognitive control of drug-seeking behavior[25-26]. METH induces abnormal activation of dopaminergic neurons within this pathway: it interferes with dopamine transporter (DAT) function to reduce dopamine (DA) reuptake from the synaptic cleft and inhibits vesicular monoamine transporter-2 (VMAT-2) to prevent DA sequestration into synaptic vesicles, ultimately leading to excessive DA accumulation and prolonged stimulation of dopamine receptors[25]. This hyperactivation reinforces drug-seeking behavior and contributes to the development of conditioned place preference (CPP), a behavioral paradigm reflecting drug reward[27]. The dysregulation of dopamine transmission alters synaptic plasticity and neuronal excitability, thereby modifying reward-related learning and memory processes essential for addiction maintenance.

Beyond dopamine, the balance between excitatory and inhibitory neurotransmission mediated by glutamate and gamma-aminobutyric acid (GABA) systems is also disrupted in METH addiction[28-29]. Within the METH-activated reward circuitry, glutamatergic signaling dysregulation is driven by aberrant DA release: elevated extracellular glutamate (Glu) levels activate N-methyl-D-aspartate receptors (NMDARs) in the NAc and PFC, triggering excessive Ca^{2+} influx that exacerbates neurotoxicity and maladaptive neural remodeling[25]. Concurrently, GABAergic inhibitory control is impaired, particularly in the VTA-NAc projection, diminishing the brain's capacity to counterbalance excitatory signals from Glu, which further enhances neuronal excitability and vulnerability to METH-induced neuroadaptations[26]. Concurrently, GABAergic inhibitory control is impaired, diminishing the brain's capacity to counterbalance excitatory signals. This imbalance contributes to heightened neuronal excitability and vulnerability to METH-induced neuroadaptations, fostering compulsive drug use and relapse[28, 30].

At the molecular level, the expression and function of neurotransmitter receptors are dynamically regulated in response to METH exposure. Alterations in receptor density, subunit composition, and downstream signaling pathways affect synaptic efficacy and neural network function[25]. For instance, changes in dopamine receptor subtypes modulate reward sensitivity, while modifications in glutamate and GABA receptor expression influence synaptic strength and plasticity[28-29, 31]. Recent research has also highlighted the role of other neurotransmitter systems, such as serotonin (5-HT), in METH addiction[32]. Within the reward circuitry, METH inhibits the 5-HT transporter (SERT) to reduce 5-HT reuptake, and this elevated extracellular 5-HT further modulates DA release in the NAc *via* 5-HT_3 receptor activation[26]. A study investigating the protein phosphatase Mg^{2+}/Mn^{2+}-dependent 1F (PPM1F) in dorsal raphe nucleus (DRN) 5-HT neurons demonstrated that METH decreases PPM1F expression, which correlates with enhanced CPP behaviors. PPM1F modulates key

components of the serotonergic system, including tryptophan hydroxylase 2 (TPH2) and serotonin transporter (SERT), leading to reduced 5-HT levels and impaired serotonergic neurotransmission[33]. These findings suggest that serotonergic dysfunction, mediated by altered receptor and transporter regulation, contributes to METH addiction pathology and may serve as a diagnostic biomarker or therapeutic target[33].

Collectively, the aberrant activation of the dopamine system, the disrupted balance between glutamate and GABA neurotransmission, and the regulatory changes in neurotransmitter receptor expression and function constitute a complex neurochemical framework underlying METH addiction. Understanding these molecular and cellular alterations is crucial for developing targeted interventions to restore neurotransmitter system homeostasis and mitigate addictive behaviors.

1.2 Neuroinflammation and oxidative stress

Building upon neurotransmitter dysregulation, neuroinflammation and oxidative stress represent additional critical pathological mechanisms in METH addiction. METH abuse induces a complex neuroinflammatory response characterized by activation of microglia and astrocytes, leading to the release of pro-inflammatory cytokines and chemokines that exacerbate neuronal injury[34]. Molecular markers of METH-induced neuroinflammation include elevated expression of Toll-like receptor 4 (TLR4), nuclear factor kappa B (NF- κB), interleukin-1β (IL-1β), tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6) in brain regions such as the prefrontal cortex and hippocampus[34, 35]. Studies demonstrate that METH exposure upregulates TLR4 and its downstream signaling adaptor TRIF, which in turn activates NF-κB and mitogen-activated protein kinase (MAPK) pathways, culminating in increased production of inflammatory cytokines and microglial activation[34-35].

The triggering receptor expressed on myeloid cells 2 (TREM2), a microglial receptor, is also implicated in modulating METH-induced neuroinflammation, with TREM2 deficiency exacerbating microglial activation and inflammatory responses[36]. Furthermore, METH-induced neuroinflammation is linked to neuronal apoptosis and cognitive deficits, with evidence indicating that neuronal TLR4 expression contributes to neurotoxicity and that pharmacological inhibition of TLR4 signaling by agents such as Ibudilast attenuates METH-induced addictive behaviors and neuroinflammation[35, 37].

The neuroinflammatory cascade is closely intertwined with oxidative stress mechanisms. METH increases reactive oxygen species (ROS) production, mitochondrial dysfunction, and oxidative damage to lipids, proteins, and DNA, which further potentiate neuroinflammation and neuronal death[38-39]. Oxidative stress markers such as malondialdehyde (MDA), protein carbonyls, and decreased antioxidant enzyme activities (*e.g.*, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px)) are observed following METH exposure[40-41].

The interplay between oxidative stress and neuroinflammation is mediated *via* signaling pathways involving NF-κB and Nrf2, where oxidative stress activates NF-κB to promote pro-inflammatory gene expression, while Nrf2 activation exerts antioxidant and anti-inflammatory effects[42-43]. In the context of METH neurotoxicity, therapeutic strategies targeting these pathways have shown promise; for example, cannabidiol (CBD) inhibits METH-induced oxidative neurotoxicity by regulating transient receptor potential vanilloid type 1 (TRPV1)-mediated Ca^{2+} influx and oxidative stress[44]. Additionally, natural compounds such as thymoquinone and polysaccharides have demonstrated neuroprotective effects by reducing oxidative stress and neuroinflammation in METH models[45-46].

Gene expression analyses of postmortem brain tissue from chronic METH users reveal upregulation of autophagy and apoptosis-related genes alongside markers of oxidative stress and reduced neurotrophic factors, underscoring the multifactorial nature of METH-induced neurodegeneration[47-48]. Moreover, the gut-brain axis has emerged as a novel contributor to METH-induced neuroinflammation, where METH-induced gut dysbiosis increases intestinal permeability and systemic inflammation, further amplifying neuroinflammatory responses[49]. Exercise and pharmacological interventions that modulate neuroinflammation and oxidative stress pathways have shown neuroprotective potential in METH models, improving cognitive function and reducing neuroinflammation[50-51]. Collectively, these findings highlight that METH-induced neuroinflammation and oxidative stress are closely linked processes involving multiple molecular markers and signaling pathways, which constitute promising targets for diagnostic and therapeutic strategies in METH addiction and neurotoxicity.

1.3 Epigenetic mechanisms

Methamphetamine (METH), a highly addictive illegal psychostimulant, exhibits complex addictive mechanisms that involve the activation of multiple neurotransmitter systems, including dopamine, glutamate, norepinephrine, and serotonin, thereby affecting the function and structure of multiple brain regions in the central nervous system. In recent years,

an increasing number of studies have demonstrated that epigenetic mechanisms play a crucial role in regulating METH addiction[2]. These mechanisms mainly include DNA methylation, histone modifications (*e.g.*, acetylation and methylation), and regulation by non-coding RNAs (microRNAs and long non-coding RNAs), which are involved in the dynamic regulation of gene expression and changes in neural plasticity[2, 25].

DNA methylation is a key focus in epigenetic research on METH addiction. METH exposure can induce changes in DNA methylation or hydroxymethylation in the promoter regions of specific genes, thereby regulating the expression of genes associated with neural reward pathways. For instance, in animal models of METH self-administration, relevant genes in the nucleus accumbens shell exhibit dynamic changes in DNA hydroxymethylation, leading to upregulated mRNA expression of potassium channel-encoding genes. This is correlated with animals' compulsive METH intake behavior and sensitivity to punishment[26]. Furthermore, polymorphisms and DNA methylation levels of the SLC6A4 and COMT genes in human populations have been found to be closely associated with susceptibility to METH addiction. Epigenetic regulation of these genes may affect the metabolism and function of dopamine and serotonin, thereby influencing an individual's risk of METH addiction[52].

Histone modifications, particularly acetylation and methylation, also play important roles in METH-induced regulation of gene expression and neural adaptation. Chronic METH use can alter the histone acetylation status of key genes in brain regions such as the prefrontal cortex and striatum, promoting the transcriptional activity of addiction-related genes. For example, increased expression of the ANP32A and POU3F2 genes is accompanied by hyperacetylation of histones H3 and H4 in their promoter regions, indicating that histone acetylation is involved in the development of METH-induced behavioral sensitization[53]. The role of histone deacetylases (HDACs) in regulating these processes has also been confirmed; changes in HDAC activity may affect the expression of immediate early genes (IEGs), thereby mediating neural plasticity and addictive behaviors[54].

Non-coding RNAs, especially microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), as important molecules in epigenetic regulation, have also been found to be involved in the regulation of METH addiction. miR-31-3p regulates METH-induced conditioned place preference in the dorsal hippocampus by targeting RhoA, suggesting that miRNA-mediated signaling pathways exert regulatory effects in the neural mechanisms of METH addiction[55]. Additionally, miR-222-3p is downregulated in the nucleus accumbens shell, and its overexpression can inhibit METH-induced reward behavior, demonstrating the potential therapeutic value of miRNAs in METH addiction[56]. Emerging non-coding RNAs such as circular RNAs (circRNAs) and tRNA-derived small RNAs (tsRNAs) have also been increasingly revealed to be involved in regulating METH-induced neurotoxicity and behavioral changes. For example, circHomer1 alleviates METH-induced neurodamage by regulating the expression of the *Bbc3* gene[57], and tRF-1-32-Gly-GCC-2-M2 can target BDNF to participate in the addiction process[58].

Notably, METH-induced epigenetic changes are not limited to the individual itself but may also transmit addiction susceptibility across generations by affecting the DNA methylation status of germ cells. For example, mouse models of maternal METH exposure show enhanced behavioral sensitivity to METH in offspring, accompanied by significant changes in DNA methylation and expression of relevant genes in the nucleus accumbens shell. This suggests that epigenetic remodeling induced by METH exposure may affect the neural development and addiction risk of offspring[59]. Similarly, paternal METH exposure can alter the DNA methylation profile of sperm and affect the transcriptome expression in the prefrontal cortex of offspring, further verifying the transgenerational effects of METH-induced epigenetic changes[60].

Collectively, the epigenetic mechanisms of METH addiction form a complex, multi-level, and multi-mechanism intertwined network, involving various epigenetic processes such as DNA methylation, histone modifications, and non-coding RNA regulation. These mechanisms not only regulate the expression of addiction-related genes and the plasticity of neural circuits but also may affect an individual's addiction susceptibility and relapse risk.

2 The effects of exercise on the nervous system and its molecular basis

2.1 Mechanisms of exercise−promoted neural plasticity

The core protective effect of exercise on the nervous system stems from its promotion of neural plasticity through coordinated molecular, cellular, and network-level adaptations, which are particularly important for improving addiction-related neural dysfunction. A key molecular mediator of exercise-induced neuroplasticity is BDNF, whose expression and signaling pathways are robustly upregulated by physical activity. BDNF acts via its receptor TrkB to promote synaptogenesis, neurogenesis, and long-term

potentiation (LTP), facilitating learning and memory processes. Exercise-induced increases in peripheral lactate and endocannabinoid signaling correlate with elevated central BDNF levels and activation of downstream cascades such as MAPK-ERK, suggesting a metabolic and neuromodulatory link between muscle activity, neurotrophic support, synaptic plasticity, and neuroinflammation regulation[61-63].

Notably, exercise-regulated neural plasticity relies on peripheral-central crosstalk mediated by multiple exerkines, as illustrated in Figure 1, which summarizes how exercise-induced myokines, adipokines, hepatokines, and osteokines are released from peripheral tissues and converge on hippocampal signaling pathways to enhance BDNF expression, maintain neural progenitor cell function, and support synaptic remodeling[64]. Exercise induces the release of myokines (*e. g.*, FNDC5/irisin, CTSB), adipokines (*e. g.*, Adiponectin), hepatokines (*e. g.*, FGF21), and osteokines (*e. g.*, Osteocalcin), which cross the blood-brain barrier and converge on hippocampal signaling pathways (cAMP/PKA/CREB, AMPK, SIRT1/PGC1α, GPR158) to upregulate BDNF, maintain neural progenitor cell function, and support synaptic remodeling[64]. Additionally, exercise induces brain-derived Leptin, which exerts neuroprotection via hypothalamic-hippocampal signaling.

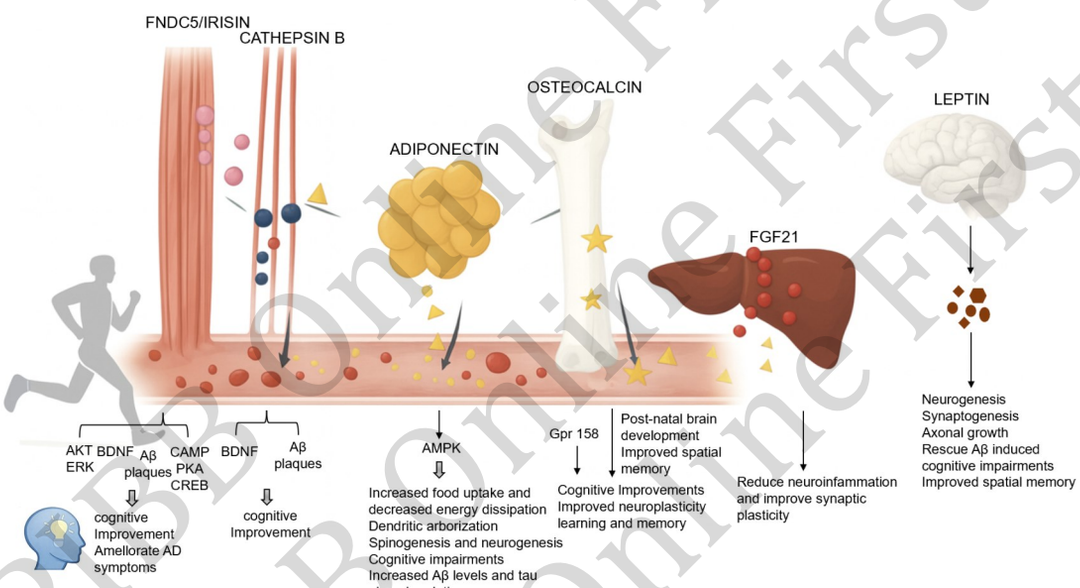

Fig. 1 Exercise–mediated release of exerkines and their effects on hippocampal function

Exercise induces release of exerkines including FNDC5/irisin, Cathepsin B, Adiponectin, Osteocalcin, and fibroblast growth factor 21 from skeletal muscle (myokines), adipose tissue (adipokines), liver (hepatokines), and bone (osteokines), respectively. These exerkines cross the blood-brain barrier to modulate the activities of important hippocampal signaling molecules, including AKT/ERK, AMP-activated protein kinase, cyclic adenosine monophosphate/protein kinase A/cAMP response element-binding protein, BDNF, and G protein-coupled receptor 158, thereby exerting exercise-induced benefits on the brain. Exercise also stimulates the synthesis and secretion of Leptin in the brain, which plays a role in neuroprotection.

At the synaptic level, exercise induces dynamic remodeling of synaptic structures and functions. This includes increased dendritic spine density, enhanced synaptic efficacy, and modulation of neurotransmitter release, which collectively strengthen neural circuits involved in cognition and motor control. Functional neuroimaging studies reveal that exercise enhances both functional and structural connectivity within key brain networks, such as the default mode network and frontoparietal circuits, which are critical for cognitive and sensorimotor functions[65-66]. Exercise also modulates synaptic plasticity in specific brain regions, including the hippocampus and anterior cingulate cortex, through mechanisms involving neurotransmitters like serotonin and endocannabinoids, which regulate synaptic strength and plasticity[67-68]. Furthermore, transcranial magnetic stimulation studies demonstrate that exercise increases corticospinal excitability and modulates cortical inhibition, reflecting enhanced neurophysiological plasticity[69-70].

Neurogenesis and neuronal repair represent additional critical components of exercise-promoted neural plasticity. Sustained physical activity stimulates adult hippocampal neurogenesis, contributing to improved cognitive function and resilience against neurodegenerative processes[71]. Exercise also facilitates regeneration after nerve

injury by promoting axonal growth, reducing inflammation, and restoring synaptic connectivity, thereby enhancing sensorimotor recovery[72]. The interplay between astrocytes, microglia, and neurons is also influenced by exercise, where astrocyte proliferation and glial-neuronal crosstalk support synaptic remodeling and neurovascular adaptations essential for plasticity[73]. Moreover, mitochondrial function modulation by exercise underpins the energetic demands of neurogenesis and synaptic remodeling, linking cellular bioenergetics with plasticity and behavioral outcomes[72, 74].

Exercise-induced neural plasticity further involves coordinated changes in gene expression, epigenetic modifications, and regulation by transcription factors and non-coding RNAs. Studies have demonstrated that physical exercise robustly increases the expression of immediate early genes (IEGs) such as ARC, c-Fos, and EGR-1 in the hippocampus, a brain region critical for cognitive function. These IEGs serve as molecular markers of neuronal activation and synaptic plasticity, facilitating changes in synaptic structure and connectivity that underlie improved cognitive performance[75]. The upregulation of BDNF, synaptotagmin, and other synaptic proteins observed in exercised animals correlates with enhanced neurogenesis and synaptic function across species, indicating a conserved mechanism of exercise-induced neural plasticity[76-77].

Epigenetic modifications are increasingly recognized as crucial mediators of exercise-induced gene expression changes in the brain. DNA methylation and histone modifications dynamically regulate the accessibility of plasticity-related genes such as *Bdnf*, influencing their transcriptional activity. For example, exercise has been shown to modulate *Bdnf* gene methylation in a site-specific and sex-dependent manner, particularly in the prefrontal cortex, which may contribute to the neuroprotective and cognitive benefits of physical activity following early-life stress[78]. In addition to DNA methylation, exercise can modulate RNA-binding proteins (RBPs) that govern mRNA stability, splicing, and translation, thereby sustaining synaptic plasticity and neural homeostasis; dysregulation of RBPs is implicated in neuropsychiatric disorders such as schizophrenia, underscoring the therapeutic potential of exercise-mediated RBP modulation[79].

Transcription factors and non-coding RNAs further orchestrate the gene expression changes underlying exercise-induced neural plasticity. Transcription factors such as Smad2, within the TGF-β signaling pathway, help balance the proliferation and maturation of hippocampal neural progenitors, while non-coding RNAs (miRNAs and long non-coding RNAs) modulate gene networks involved in synaptic remodeling, although their precise roles in exercise-induced adaptations remain to be fully defined[80]. Exercise also influences the expression of genes involved in neural stem cell maintenance and aging, such as alpha-synuclein (Snca), whose expression is downregulated in premature aging models but restored by physical activity, leading to improved neurogenesis and reduced apoptosis[81].

Collectively, these mechanisms indicate that exercise promotes neural plasticity through an integrated molecular and cellular framework in which neurotrophic factors (*e. g*., BDNF), synaptic remodeling, neurogenesis and repair processes, and gene/epigenetic regulation converge to improve brain function and resilience. The central regulatory role of BBB-crossing exerkines and brain-derived leptin further reinforces this integrative mechanism and provides a mechanistic basis for applying exercise as a therapeutic strategy in neurological and addiction-related disorders.

2.2 Exercise regulation of neurotransmitter systems

Based on exercise promoting neural plasticity, the precise regulation of neurotransmitter systems is the core molecular pathway through which exercise improves addiction-related behaviors, especially targeting the dysfunction of dopamine and glutamate systems. Exercise has been shown to modulate DA) and glutamate neurotransmission at multiple levels, including neurotransmitter release, receptor expression, and metabolism, thereby influencing neural plasticity and behavioral outcomes[17].

Exercise enhances dopaminergic signaling by increasing dopamine synthesis, release, and receptor sensitivity. For instance, high-intensity interval exercise (HIIE) has been demonstrated to elevate dopaminergic activity in brain regions associated with reward and emotion, such as the striatum and midbrain, as evidenced by increased fractional amplitude of low-frequency fluctuations (zfALFF) correlated with dopamine receptor distribution[82]. This dopaminergic upregulation is associated with improved mood, motivation, and reward processing, which are crucial in combating addictive behaviors and neurodegenerative conditions. Moreover, exercise-induced increases in dopamine transporter (DAT) expression in the striatum without damaging dopaminergic neurons suggest enhanced dopamine reuptake and synaptic regulation, supporting neuroprotective effects[83]. Exercise also modulates dopamine receptor subtypes (D1R, D2R), which are implicated in the expression of BDNF and TrkB activation, further promoting synaptic plasticity and antidepressant effects[84].

Regarding the glutamatergic system, exercise influences glutamate levels and receptor function, which are essential for excitatory neurotransmission and synaptic plasticity. Acute strenuous exercise has been shown to depress glutamate levels, contributing to the maintenance of excitatory/inhibitory balance and neuroprotection in seizure models[85]. Additionally, exercise increases the expression of glutamic acid decarboxylase (GAD65/67), enzymes responsible for converting glutamate to gamma-aminobutyric acid (GABA), thereby enhancing inhibitory neurotransmission and preventing excitotoxicity[86]. This modulation of glutamate and GABA systems by exercise is crucial for cognitive enhancement and mood regulation.

Exercise also affects neurotransmitter receptor expression. For example, increased expression of 5-HT receptors (5-HT1A, 5-HT2) and muscarinic receptors (M5) following exercise contributes to antidepressant-like effects and cognitive improvements[84]. Furthermore, exercise elevates GABA_A receptor subunits (γ2 and α2), which play roles in reducing neurodegenerative processes and inflammation[84]. These receptor-level changes facilitate enhanced neurotransmission and synaptic efficacy.

At the metabolic level, exercise influences neurotransmitter metabolism and intracellular vesicle chemistry. Single-vesicle electrochemistry studies reveal that exercise enhances neurotransmitter storage capacity within vesicles, increases release quantity, and alters exocytosis dynamics, likely mediated by upregulation of exocytosis-associated proteins and increased calcium influx[87]. Such changes optimize synaptic transmission efficiency and plasticity.

Exercise-induced modulation of amino acid neurotransmitters, including tyrosine, phenylalanine, glutamine, and GABA, also contributes to improved neuropsychological performance post-exercise, highlighting the metabolic underpinnings of neurotransmitter regulation[88-89]. Moreover, exercise impacts systemic and central neurotransmitter levels, such as acetylcholine, norepinephrine, serotonin, and dopamine, which are often decreased in neurodegenerative and vascular dementia models but restored by exercise interventions[90-91].

In summary, exercise exerts multifaceted regulatory effects on neurotransmitter systems by enhancing neurotransmitter synthesis and release, modulating receptor expression, and altering neurotransmitter metabolism. These changes collectively contribute to improved cognitive function, mood regulation, neuroprotection, and attenuation of addictive behaviors, and they interact with neurotrophic signaling to further strengthen brain plasticity and function.

2.3 Regulation of inflammation and oxidative stress by exercise

In addition to regulating neural plasticity and neurotransmitters, the inhibitory effect of exercise on inflammation and oxidative stress is another key molecular mechanism by which it counteracts methamphetamine addiction-related neurotoxicity. At the molecular level, exercise reduces neuroinflammation through multiple intertwined mechanisms. One key pathway involves the downregulation of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, which are often elevated in pathological states. For example, aerobic exercise has been shown to attenuate chronic allergic airway inflammation by modulating the circMETTL9/EIF4A3/IGFBP3 axis, thereby reducing inflammatory and oxidative stress markers[92]. Similarly, exercise interventions in obese and metabolic disorder models demonstrate reductions in systemic and tissue-specific inflammation, including decreased expression of NLRP3 inflammasome components and related cytokines IL-1β and IL-18, highlighting exercise's capacity to downregulate innate immune activation[93-94].

The anti-inflammatory effects of exercise are further mediated by molecular regulators such as miRNAs; for instance, miR-221-3p is diminished by aerobic exercise, leading to improved macrophage polarization and reduced chronic inflammation in skeletal muscle[95]. Moreover, exercise-induced activation of transcription factors like nuclear factor erythroid 2-related factor 2 (Nrf2) enhances the endogenous antioxidant defense system, thereby mitigating oxidative stress and its downstream inflammatory sequelae[96-97]. This activation promotes the expression of antioxidant enzymes such as SOD, CAT, and GSH-Px, which neutralize ROS generated during metabolic and pathological processes. Exercise also modulates redox-sensitive signaling pathways, including AMPK and PI3K/AKT, which coordinate cellular responses to oxidative stress and inflammation, as seen in models of obesity, cardiomyopathy and ischemic stroke[98-99].

Importantly, the intensity and duration of exercise critically influence these molecular responses; moderate-intensity aerobic exercise generally promotes anti-inflammatory and antioxidant effects, whereas excessive high-intensity exercise may transiently elevate oxidative stress and inflammatory markers[93, 100]. Notably, exercise-induced exerkines also regulate inflammation and oxidative stress: FGF21 inhibits hippocampal pro-inflammatory factor release *via* SIRT1, and Adiponectin reduces microglial activation *via* AMPK, synergizing with

their role in neural plasticity to maintain central homeostasis[64]. Exercise-induced changes in anti-inflammatory genes and exerkines further contribute to systemic inflammation resolution and oxidative balance[101].

Collectively, these molecular mechanisms underscore the role of exercise as a potent modulator of neuroinflammation and oxidative stress, offering therapeutic potential for conditions characterized by chronic inflammation and oxidative damage, including methamphetamine addiction.

3 Mechanisms of regulation of exercise-induced biomarkers

3.1 Screening and identification of key biomarkers

To analyze the regulatory mechanisms of exercise on biomarkers, the primary step is to screen and identify key biomarkers that respond to exercise and are closely associated with the pathology of METH addiction. High-throughput sequencing technologies, such as transcriptome sequencing (RNA-seq), have been extensively employed to screen key exercise-responsive biomarkers and characterize gene expression profiles associated with addiction states. For instance, in a study investigating treadmill exercise intervention in METH-addicted mice, transcriptome sequencing revealed 316 differentially expressed genes (DEGs) between METH-treated and control groups, and 156 DEGs between exercise-treated and METH groups, these DEGs serve as potential key biomarkers that highlight the molecular impact of exercise on addiction-related gene expression[102]. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of these DEGs provided functional annotations for key biomarkers and implicated critical signaling pathways such as PI3K-Akt, mTOR, and Wnt, which are known to regulate neuronal plasticity and cognitive function. Cross-comparative analyses identified 43 DEGs modulated by exercise in METH-addicted mice, these are core key biomarkers including genes like NFKBIA and CXCL12, which are involved in inflammatory and neuroimmune responses, suggesting that exercise may exert neuroprotective effects through modulation of these pathways[102].

Expression profiling of addiction-related biomarkers has also been advanced by miRNA studies, which have identified specific miRNAs as non-coding RNA-based key biomarkers differentially expressed in METH addiction. For example, let-7b-3p was found to be significantly overexpressed in brain regions implicated in addiction, such as the ventral tegmental area and nucleus accumbens, demonstrating its potential as a diagnostic and therapeutic biomarker for METH addiction[103]. Moreover, epigenetic regulatory mechanisms involving DNA methylation, histone modifications, and non-coding RNAs have been shown to regulate the expression of biomarkers associated with neuroreward pathways and addiction susceptibility, underscoring the complexity of gene regulation in METH addiction[25].

The interplay between exercise and addiction-related biomarkers expression is further elucidated by studies demonstrating that exercise influences gene networks involved in neurotransmitter systems. For example, PPM1F, expressed in dorsal raphe nucleus serotonergic neurons, modulates METH-induced conditioned place preference behaviors by regulating serotonin synthesis and transport genes such as tryptophan hydroxylase 2 (TPH2) and serotonin transporter (SERT). Exercise-induced modulation of such key biomarkers may underlie the observed improvements in cognitive function and addiction phenotypes[33]. Notably, METH disrupts the expression of biomarkers in dopaminergic and glutamatergic systems, which are two core pathways in addiction, and exercise reverses these dysregulations, as summarized in Figure 2, where chronic exercise restores D1/D2 receptor balance, normalizes DAT and GLT1 expression, and attenuates aberrant glutamate release[17]. Specifically, METH upregulates the dopamine D1 receptor gene (*DRD1*), downregulates the dopamine D2 receptor gene (*DRD2*) and dopamine transporter gene (*SLC6A3*, encoding DAT), leading to reduced basal dopamine levels; exercise restores DRD1/DRD2 balance, normalizes SLC6A3 expression, and elevates dopamine[17]. For glutamatergic biomarkers, METH increases the expression of genes encoding NMDAR1 (*GRIN1*), AMPAR1 (*GRIA1*), and mGluR5 (*GRM5*), decreases the expression of genes encoding mGluR2 (*GRM2*) and glutamate transporter GLT1 (*SLC1A2*), and triggers excessive cue-induced glutamate release; exercise corrects these abnormalities to inhibit aberrant glutamate transmission[17]. Additionally, systemic inflammation and gut-immune-brain axis alterations induced by METH have been linked to changes in gene expression related to tight junction proteins and inflammatory biomarkers, which exercise may counteract, thereby contributing to recovery[104].

To comprehensively understand the cross-regulatory networks between exercise-responsive and addiction-related biomarkers, integrative bioinformatics approaches combining differential gene expression data, pathway enrichment analyses, and gene co-expression networks are employed. These analyses facilitate the identification of hub genes and molecular modules that are co-regulated by exercise and METH exposure. For instance, transcriptomic

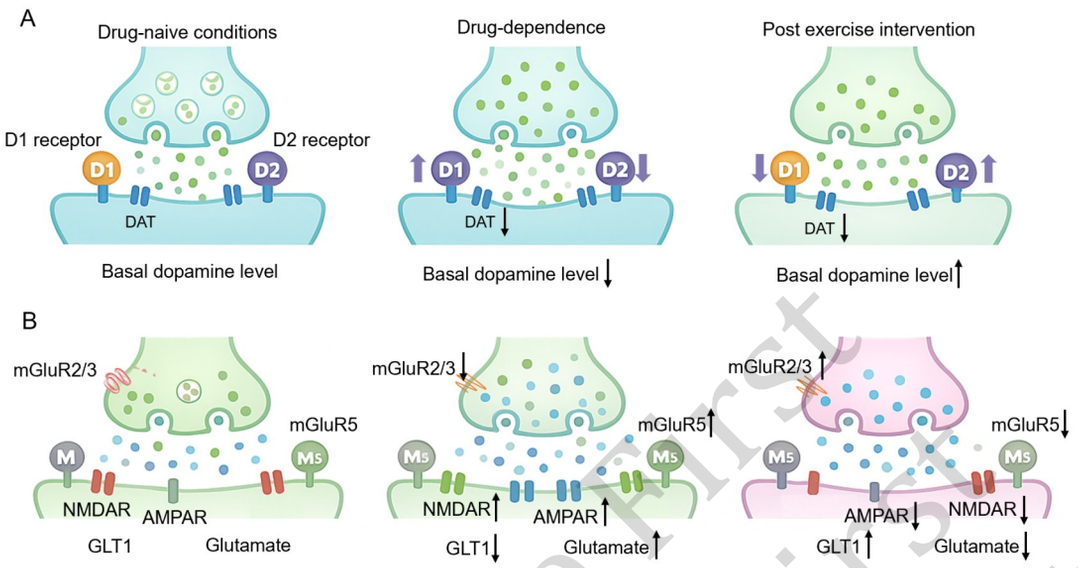

Fig. 2　Neuroadaptations in dopaminergic and glutamatergic systems following chronic addictive substance exposure and exercise intervention

(a) Dopaminergic system, under drug-naive conditions, the homeostasis is maintained with normal densities of D1 receptors, D2 receptors, dopamine transporters (DAT), and basal dopamine levels in the reward circuit. During drug dependence, there are altered densities of dopamine receptors (increased D1 receptors and decreased D2 receptors), reduced DAT density, and a decreased basal dopamine level. After chronic exercise intervention, the disturbed homeostasis is normalized by decreased D1 receptors, increased D2 receptor density, reduced DAT density, and elevated basal synaptic dopamine level. (b) Glutamatergic system, under drug-naive conditions, the system exhibits normal levels of pre- and postsynaptic receptors (NMDAR, presynaptic autoinhibitory mGluR2/3, postsynaptic mGluR5, AMPAR), glial glutamate transporter GLT1, and glutamate levels during cue induction. In the drug-dependent state, there are changes in synaptic plasticity (increased NMDAR and AMPAR), reduced presynaptic mGluR2/3, increased postsynaptic mGluR5, decreased GLT1 density, and an overt burst release of glutamate during cue induction. Following chronic exercise intervention, presynaptic mGluR2/3 density increases, postsynaptic mGluR5 density decreases, GLT1 density increases, synaptic plasticity (NMDAR and AMPAR) decreases, and brain glutamate levels reduce, thereby normalizing the disrupted homeostasis and attenuating responses to addiction-related cues.

meta-analyses in other biological systems have successfully delineated transcription modules responsive to stress and exercise, enabling the discrimination of pathogen-specific responses from general inflammation and stress[105]. Applying similar strategies to addiction research can reveal overlapping and distinct gene sets modulated by exercise and METH, providing insights into molecular targets for intervention.

In summary, the application of high-throughput sequencing technologies has enabled the identification of key biomarkers responsive to exercise and involved in METH addiction. Expression profiling and network analyses have uncovered complex regulatory interactions between exercise and addiction-related biomarkers, involving signaling pathways, epigenetic mechanisms, and neurotransmitter systems. These findings lay the groundwork for developing molecular diagnostic tools and therapeutic strategies targeting exercise-mediated modulation of addiction. Further validation of candidate biomarkers and elucidation of their functional roles remain essential to translate these molecular insights into clinical applications.

3.2　Epigenetic regulatory mechanisms

After identifying key biomarkers, epigenetic modification, as the core molecular mechanism by which exercise regulates the expression of these biomarkers, is a critical bridge connecting exercise stimuli to changes in gene expression. Exercise-induced epigenetic modifications encompass DNA methylation, histone post-translational modifications, and non-coding RNA-mediated regulation, which collectively orchestrate transcriptional programs essential for cellular and systemic responses[106]. In skeletal muscle, resistance exercise preferentially stimulates ribosome biogenesis through epigenetic changes such as rDNA enhancer methylation alterations, independent of promoter methylation, highlighting the nuanced locus-specific epigenetic remodeling that supports hypertrophic adaptation[107]. Similarly, exercise modulates DNA methylation patterns in various tissues, including intervertebral discs, where physical activity attenuates low back pain and alters expression of epigenetic regulatory genes such as Mecp2, with sex-specific effects observed[108]. These findings underscore the dynamic

and tissue-specific nature of DNA methylation in response to exercise stimuli.

Histone modifications, particularly acetylation and deacetylation, are pivotal in exercise-induced gene regulation. For instance, exercise elevates histone acetylation levels (H3 and H4) in the motor cortex post-intracerebral hemorrhage, enhancing neuroplasticity and functional recovery, an effect that can be pharmacologically mimicked by HDAC inhibitors[109]. In skeletal muscle, HDAC5 acts as a negative regulator of IL-6 (a key exercise-induced inflammatory biomarker) secretion and insulin action, with exercise-induced HDAC5 deficiency leading to increased histone acetylation at the Il6 promoter, thereby promoting glucose metabolism and systemic glucose tolerance[110]. These epigenetic mechanisms involving histone modifications facilitate rapid and reversible changes in chromatin accessibility, enabling exercise to fine-tune gene expression linked to metabolic and neuroplastic adaptations.

Non-coding RNAs, including miRNAs and lncRNAs, constitute another layer of epigenetic regulation modulated by exercise. Exercise influences the expression of various ncRNAs that regulate neuroplasticity, cardiac function, and metabolic homeostasis. For example, aerobic exercise modulates cardioepigenetic landscapes by altering miRNAs and lncRNAs implicated in vascular inflammation and cardiac remodeling, thereby contributing to cardiovascular health benefits[111]. In the context of addiction, miRNAs such as miR-31-3p and miR-222-3p in the nucleus accumbens modulate methamphetamine-induced conditioned place preference by targeting genes involved in synaptic plasticity and reward pathways, highlighting the therapeutic potential of targeting ncRNA-mediated epigenetic mechanisms[55, 56]. Notably, exercise may regulate the expression of dopaminergic and glutamatergic biomarkers (DRD2 and GRM2) *via* non-coding RNAs, which aligns with the exercise-induced restoration of DRD2, mGluR2/3, and GLT1 levels in dopaminergic and glutamatergic circuits, as depicted in Figure 2[17]. Additionally, exercise-induced changes in RNA modifications, such as m6A methylation, have been observed in the hippocampus and hypothalamus, linked to downregulation of the FTO demethylase, suggesting that exercise can epigenetically regulate RNA metabolism to influence brain function[112].

Collectively, these epigenetic modifications induced by exercise impact biomarkers associated with addiction and neuroplasticity. DNA methylation changes can alter the expression of addiction-related biomarkers, as evidenced by methamphetamine exposure studies showing altered DNA methylation and hydroxymethylation patterns in brain regions critical for reward and addiction behaviors[26, 59]. Histone modifications regulate immediate early gene expression and chromatin remodeling necessary for drug-induced neuronal plasticity, with HDAC inhibitors demonstrating potential to modulate these processes[54]. Non-coding RNAs further modulate gene expression networks underlying addiction vulnerability and resilience. Importantly, exercise-mediated epigenetic remodeling may counteract maladaptive epigenetic states induced by drugs of abuse, offering a promising avenue for addiction diagnosis and therapy. Understanding the interplay between exercise, epigenetic regulation, and addiction-related biomarkers expression provides a mechanistic framework for developing non-pharmacological interventions and epigenetic therapeutics targeting methamphetamine addiction.

3.3 Regulation of signal transduction pathways

Physical exercise activates key signal transduction pathways such as MAPK and PI3K/Akt, which are crucial mediators linking extracellular stimuli to intracellular gene expression changes. The MAPK pathway is well-recognized for its role in regulating cellular responses to stress, growth factors, and inflammatory signals, while the PI3K/Akt pathway is pivotal for cell survival, metabolism, and synaptic plasticity. Notably, these two pathways do not operate in isolation but form an interconnected regulatory network with the Wnt pathway, and their intricate cross-talk and synergistic interactions are indispensable for precisely modulating cellular adaptive responses to exercise. Mechanistically, the MAPK and PI3K/Akt pathways can mutually regulate each other's activation status through upstream receptor crosstalk and downstream signaling molecules: for instance, PI3K/Akt signaling can phosphorylate and modulate the activity of key kinases in the MAPK pathway, while activated MAPK subtypes (*e. g*., ERK1/2, p38) can also influence the PI3K/Akt cascade by regulating the expression or function of its upstream components[113]. Additionally, both pathways directly interact with the Wnt pathway, primarily through the regulation of glycogen synthase kinase 3β (GSK-3β) and β-catenin, core components of the Wnt signaling cascade. Such synergistic effects of the three pathways in cell fate regulation have been validated in multiple disease models, for example, Strophanthidin, a natural cardiac glycoside, induces cell cycle arrest and apoptosis in various human cancer cells by inhibiting the expression of key proteins in the MAPK, PI3K/Akt/mTOR, and Wnt/β-catenin pathways[113]. In prostate cancer, the PI3K-AKT-mTOR pathway exhibits significant cross-regulation with the MAPK and Wnt

signaling pathways, and their collaboration promotes tumor progression and resistance to targeted therapy[114], while in the differentiation of corneal endothelial-like cells, the cross-network formed by these three pathways is essential for determining cell fate[115]. Exercise-induced ROS act as signaling molecules that modulate these pathways without causing excessive oxidative damage, as shown by the selective antioxidant effects of hydrogen gas inhalation during exercise, which preserved mitochondrial biogenesis signaling through PGC-1α activation without blunting beneficial ROS-mediated signals[116]. Similarly, three-dimensional culture studies have demonstrated that activation of MAPK signaling enhances stemness and differentiation capacities, emphasizing the pathway's role in cellular adaptation and regeneration[117].

In the context of METH addiction, exercise modulates these signaling cascades to exert neuroprotective and behavioral effects. For instance, treadmill exercise suppressed METH-induced hyperactivity by downregulating striatal glutamatergic signaling components such as NMDAr1 and MAPKs, while increasing inhibitory phosphorylation of GSK-3β, a downstream target of PI3K/Akt that also serves as a critical negative regulator of the Wnt pathway by mediating β-catenin ubiquitination and degradation. This exercise-induced phosphorylation of GSK-3β not only inhibits its pro-apoptotic activity *via* the PI3K/Akt pathway but also stabilizes β-catenin to activate Wnt-mediated neuroprotective gene expression. Furthermore, MAPK subtypes like ERK1/2 can phosphorylate GSK-3β at distinct residues, further fine-tuning the balance between PI3K/Akt and Wnt pathway activation[118-119]. Mutations and abnormal expressions of these three pathways are widely involved in pathological processes: mutations in the Wnt/β-catenin pathway (*e. g*., APC and FAT1) in Hürthle cell carcinoma cooperate with the activation of the MAPK and PI3K-AKT-mTOR pathways to promote tumorigenesis[120], and inhibiting these three pathways can effectively suppress the proliferation and migration of osteosarcoma cells[121]. Additionally, factors such as CCL2 can simultaneously activate the MAPK/ERK1/2, PI3K/Akt, and Wnt/β-catenin pathways to promote cell proliferation and angiogenesis, reflecting the synergistic amplification effect of the three pathways under pathological conditions[122], thereby reducing neurotoxicity and behavioral sensitization[123]. This regulatory effect is linked to exercise-mediated correction of biomarker expression in glutamatergic and dopaminergic systems. METH-induced overactivation of NMDAr1 (encoded by GRIN1) triggers hyperactivity of the Ca^{2+}-CREB pathway, and exercise downregulates GRIN1 (a key glutamatergic biomarker) to inhibit this aberrant signaling[17]. For the dopaminergic system, exercise-restored DRD2 expression (a core dopaminergic biomarker) enhances inhibitory regulation of the cAMP-PKA pathway, which is hyperactivated in METH addiction, thereby reducing addictive behaviors [17]. Transcriptomic analyses further revealed that exercise intervention in METH-addicted mice significantly enriched DEGs associated with the PI3K-Akt, mTOR, and Wnt pathways[102], highlighting the integrated synergistic network of these three pathways in mediating exercise-induced recovery of cognitive functions. Specifically, the PI3K-Akt pathway can reinforce Wnt pathway activation by sustaining GSK-3β phosphorylation, while MAPK signaling modulates the nuclear translocation and transcriptional efficiency of β-catenin, the key effector of the Wnt pathway. Conversely, the Wnt pathway can feedback-regulate MAPK and PI3K-Akt pathways by modulating the expression of their upstream ligands (*e. g*., growth factors) or receptors, forming a reciprocal regulatory loop that amplifies neuroprotective effects and restores cognitive function[124]. Exercise-induced regulation of these three pathways has also been confirmed in other physiological processes: exercise training modulates PI3K/Akt, MAPK, and Wnt/β-catenin signals to promote neuroprotection, muscle adaptation, and bone metabolism[124-125], and during exercise-induced fatigue and bone repair, these pathways ensure tissue functional recovery through complex cross-regulation of cell proliferation, apoptosis, and differentiation[122, 126]. Moreover, non-coding RNAs such as miRNAs and circRNAs act as regulators in these three pathways, participating in the fine-tuning of signals and the feedback mechanisms between pathways[127-128].

The coupling between signal transduction and gene expression regulation is exemplified by the activation of transcription factors downstream of these pathways, such as PGC-1α in mitochondrial biogenesis and β-catenin in hippocampal neurogenesis, which are upregulated by exercise to counteract METH-induced impairments[129]. This coupling is further strengthened by the cross-regulatory interactions among MAPK, PI3K/Akt, and Wnt pathways: for example, exercise-induced PI3K/Akt activation not only directly promotes cell survival but also synergizes with Wnt/β-catenin signaling to enhance hippocampal neurogenesis by maintaining β-catenin stability[124-125], while MAPK signaling modulates the transcription of PGC-1α and β-catenin target genes involved in neuroplasticity and mitochondrial function[118]. Additionally, these pathways converge on common downstream targets

such as CREB, where their combined activation optimizes the transcription of genes related to cellular repair and functional recovery[122, 126]. Moreover, the Wnt pathway, modulated by exercise and gene therapy approaches, contributes to neuroprotection and functional recovery by regulating gene expression involved in cell proliferation and differentiation[130]. In addiction-related behaviors, the basolateral amygdala-prelimbic cortex circuit's activity is modulated *via* AMPK signaling downstream of adiponectin receptor 1, a mechanism influenced by exercise that suppresses methamphetamine-associated memory formation [131]. Collectively, these findings underscore that exercise-induced activation of MAPK and PI3K/Akt pathways, together with their synergistic cross-talk with the Wnt pathway, together with their synergistic cross-talk with the Wnt pathway, orchestrates gene expression programs that promote neuroplasticity, reduce neuroinflammation, and ameliorate addiction-related behaviors. The dynamic interplay and reciprocal regulation among these three signaling pathways form a core mechanistic basis for the therapeutic effects of exercise in METH addiction, offering multiple potential targets for intervention strategies.

4 Application of exercise−mediated biomarkers in the diagnosis of methamphetamine addiction

4.1 Potential of exercise−mediated biomarkers as diagnostic indicators

The potential of biomarkers (including gene expression-related molecules) in the diagnosis and prognosis of addiction, including METH addiction, is increasingly supported by evidence linking their expression changes to addiction severity and clinical outcomes. Biomarker alterations often reflect underlying pathophysiological processes, and their correlation with addiction severity can provide a molecular basis for objective assessment. For instance, in various cancers such as hepatocellular carcinoma (HCC), colorectal cancer, and small cell lung cancer, specific gene signatures have been identified that correlate strongly with disease progression and patient survival, demonstrating the feasibility of using gene expression profiles as prognostic biomarkers[132-134]. Similarly, in addiction research, metabolic and immune-related gene expression changes have been reported to associate with addiction states and treatment responses. For METH addiction, studies have shown that exercise interventions can modulate metabolic profiles, including the regulation of amino acid metabolism-related genes, which correlate with improved clinical symptoms such as reduced anxiety and cognitive impairment[9, 135]. These findings suggest that biomarkers involved in metabolic and immune pathways may serve as reliable indicators of addiction severity.

Detection of biomarkers in both peripheral blood and brain tissues is critical for developing minimally invasive diagnostic tools. Advances in molecular techniques such as quantitative real-time PCR (qRT-PCR), RNA sequencing, and single-cell RNA sequencing (scRNA-seq) have enabled sensitive and specific detection of biomarkers (gene expression) changes in blood samples, which often reflect central nervous system alterations. For example, in systemic diseases like sepsis and cancer, peripheral blood gene expression signatures have been successfully used to predict disease states and outcomes[136-137]. In addiction contexts, peripheral blood metabolomics and transcriptomics have revealed biomarkers that mirror brain changes induced by drug exposure and therapeutic interventions[9, 135]. Moreover, scRNA-seq techniques allow the identification of cell type-specific biomarkers, enhancing the resolution of biomarker discovery and enabling the distinction of heterogeneous cell populations relevant to addiction pathophysiology[138-139]. These methods collectively facilitate the translation of brain molecular changes into accessible peripheral biomarkers.

Constructing and validating multi-gene diagnostic models enhances the robustness and predictive accuracy of biomarker-based diagnosis and prognosis. A dedicated workflow for METH addiction biomarker identification (Figure 3) includes clinical cohort grouping (healthy controls, current patients, former patients), hair follicle sample preparation and RNA sequencing, multivariate analysis for differential gene screening, and functional annotation[140]. This workflow reclassifies patients into non-recovered (NR) and almost-recovered (AR) groups based on gene expression profiles, providing a molecular basis for stratifying addiction severity and supporting multi-gene model construction[140]. Multi-gene signatures integrate the expression patterns of several biomarkers, capturing the complexity of biological processes involved in addiction and improving discrimination between disease states. In oncology, multi-gene prognostic models have been developed using machine learning algorithms such as LASSO regression, random forest, and support vector machines, demonstrating high specificity and sensitivity in predicting patient outcomes[132-134]. Similar approaches have been applied in immune-related diseases and sepsis, where gene signatures predict disease progression and therapeutic responses[136, 141].

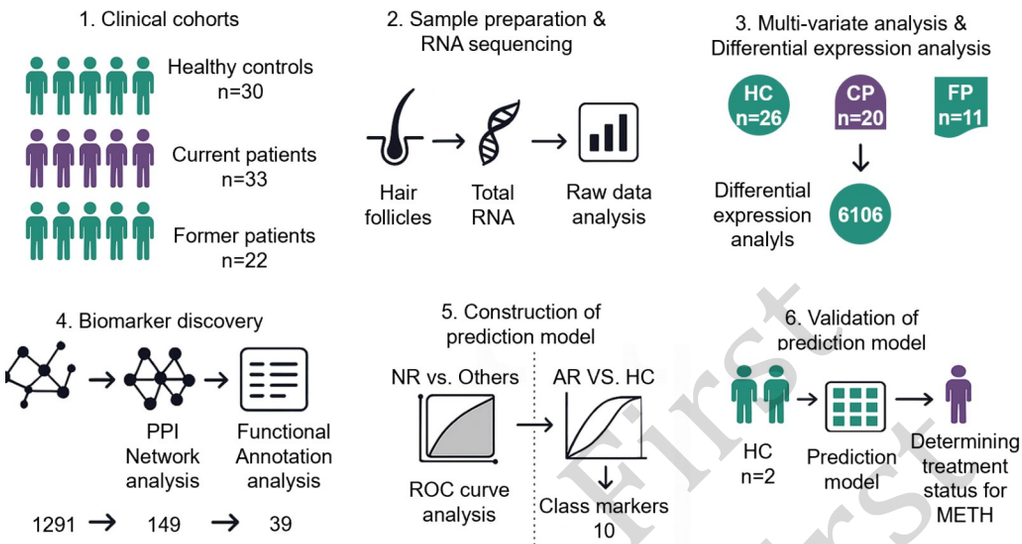

Fig. 3 Workflow for identifying biomarkers of methamphetamine use disorder diagnosis

In the study of METH addiction, the two-stage multi-gene diagnostic model constructed based on the aforementioned workflow (Figure 4) has exhibited excellent diagnostic performance: the first stage employs five biomarkers (PSMA2, RAC3, PPP1R12 A, DVL1, SUFU) to distinguish NR patients from other populations, achieving an area under the receiver operating characteristic curve (*AUC*) of 1.0 and a prediction accuracy of 98.7%; the second stage uses another five biomarkers (APC2, KLC3, NDUFA4, FADD, APOE) to differentiate AR patients from healthy controls, with an *AUC* of 0.907 and a prediction accuracy of 81.3%[140]. In addiction research, integrating gene expression data with clinical and metabolic parameters through computational models can identify gene panels that serve as biomarkers for diagnosis and monitoring treatment efficacy, including exercise interventions[9, 135]. Validation of these models in independent cohorts and across different sample types (blood and brain tissue) is essential to ensure clinical utility and generalizability.

In summary, the potential of biomarkers (including gene expression-related molecules) for methamphetamine addiction diagnosis and treatment lies in their demonstrated correlation with addiction severity, detectability in accessible tissues such as blood, and the enhanced predictive power of multi-biomarkers diagnostic models. Leveraging advanced molecular detection techniques and computational modeling can facilitate the development of reliable, non-invasive biomarkers, ultimately improving personalized management and therapeutic outcomes in METH addiction.

4.2 Dynamic monitoring of gene expression before and after exercise intervention

The dynamic monitoring of gene expression before and after exercise intervention provides critical insights into the temporal regulation of exercise-responsive genes, their correlation with behavioral outcomes, and the identification of molecular diagnostic markers that reflect the efficacy of exercise as a therapeutic modality. Exercise induces time-dependent changes in the expression of key biomarkers, such as BDNF, which plays a pivotal role in neuroplasticity and addiction recovery. Using *in vivo* bioluminescence imaging in Bdnf-Luc mice, it has been demonstrated that a single bout of treadmill exercise transiently elevates BDNF expression in the brain, peaking approximately 1 – 3 h post-exercise with a sustained, albeit diminished, increase lasting up to 24 h[142]. Repeated exercise regimens further modulate this temporal pattern of BDNF expression, accelerating the onset of BDNF upregulation after 2 weeks of training, although this effect plateaus by 4 weeks, indicating an adaptive modulation of biomarker sensitivity to exercise stimuli[143]. Such temporal dynamics underscore the importance of precise timing in biomarker monitoring to capture peak changes relevant to therapeutic outcomes.

Beyond BDNF, transcriptomic analyses of peripheral blood leukocytes reveal that exercise induces robust and dynamic changes in immune-related biomarker changes, with distinct sets of differentially expressed biomarker changes (genes) identified immediately post-exercise and at 4 hours post-exercise, but not at 20 hours, reflecting a transient immune activation and resolution pattern[144].

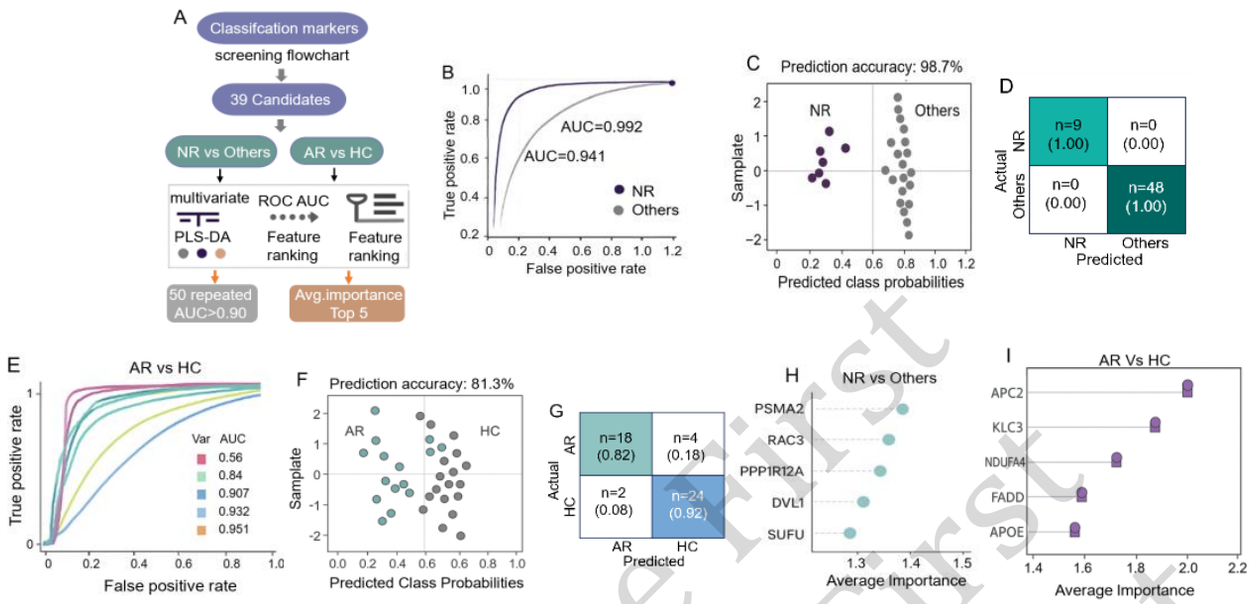

Fig. 4　Construction of the two−stage methamphetamine use disorder prediction model

（a） Flowchart of multivariate receiver operating characteristic analysis for biomarker screening. （b－d） First-stage model （distinguishing NR from others）： receiver operating characteristic curve with area under the curve （*AUC*） =1.0， prediction accuracy=98.7%， and confusion matrix. （e－g） Second-stage model （distinguishing AR from healthy controls）： receiver operating characteristic curve with *AUC*=0.907， prediction accuracy=81.3%， and confusion matrix. （h，j） Top 5 biomarkers for each stage ranked by importance.

Among these, hub genes such as *S100A12*, *FCGR3B*, and *MMP9* have been validated as potential biomarkers for monitoring exercise and training processes, suggesting their utility in molecular diagnostics. This immune gene expression profile correlates with improvements in agility and pain attenuation in clinical contexts, such as back pain therapy, where epigenetic modifications and proteomic changes in easily accessible tissues (*e. g.*, buccal swabs and plasma) align with behavioral improvements, highlighting the interplay between molecular and functional responses to exercise[145].

miRNAs, small noncoding RNAs that regulate post-transcriptional gene expression, exhibit both acute and sustained changes in response to exercise, with some miRNAs maintaining elevated expression even after exercise cessation, indicating lasting epigenetic reprogramming[146]. Circulating miRNAs also serve as promising non-invasive biomarkers reflecting systemic adaptations to physical activity, although their exercise-dependent regulation remains incompletely characterized[147]. lncRNAs, such as HOTTIP, show significant upregulation following chronic exercise and correlate with training adaptation, providing additional molecular targets for monitoring exercise effects[148].

Importantly, the association between gene expression changes and behavioral indices has been substantiated in addiction models. For example, in METH-addicted mice, moderate exercise intervention accelerates recovery as evidenced by shortened conditioned place preference durations, with transcriptomic profiling revealing 12 differentially expressed genes in the hippocampus linked to immune response pathways, which correlate with improved behavioral outcomes[8]. Similarly, treadmill exercise ameliorates cognitive impairments induced by METH, with transcriptomic analyses implicating key signaling pathways such as PI3K-Akt, mTOR, and Wnt, and identifying genes like NFKBIA and CXCL12 as mediators of exercise-induced neuroprotection[102]. These findings demonstrate that molecular changes in gene expression not only reflect but likely contribute to behavioral recovery, thus serving as valuable diagnostic indicators.

Molecular diagnostic markers derived from gene expression profiles enable objective evaluation of exercise intervention efficacy. For instance, the measurement of BDNF expression dynamics via non-invasive imaging techniques offers a direct readout of neuroplastic adaptations[142]. In peripheral tissues, integrated molecular profiling encompassing epigenetic modifications, transcriptomics, and proteomics can stratify individuals according to therapy status and health state, providing a comprehensive molecular diagnostic framework[145]. Furthermore, gene expression-based biological age clocks, such as the gene expression-based age monitoring Clock (*GamC*), demonstrate the capacity to reflect cardiovascular health and respond modestly

to exercise interventions, underscoring the potential of gene expression biomarkers in monitoring physiological adaptations[149].

In summary, dynamic monitoring of gene expression before and after exercise reveals time-dependent regulation of key genes and pathways involved in neuroplasticity, immune function, metabolism, and epigenetic remodeling. These molecular changes correlate with behavioral improvements and can be harnessed as molecular diagnostic indicators to objectively assess the efficacy of exercise interventions in conditions such as methamphetamine addiction. The integration of transcriptomic, epigenetic, and proteomic data from accessible tissues facilitates the development of comprehensive biomarkers that can guide personalized exercise-based therapies and enhance rehabilitation outcomes.

4.3 Clinical application challenges and prospects

4.3.1 Sensitivity and specificity issues in biomarker detection

The clinical application of exercise-mediated biomarker detection in METH addiction faces significant challenges related to the sensitivity and specificity of these biomarkers. Metabolomic studies have identified numerous metabolites altered by METH use and modulated by exercise interventions, such as glutamate, GABA, l-tryptophan, and uric acid, which show promise as potential diagnostic and therapeutic markers[9, 135]. However, the heterogeneity of metabolic responses and the overlap of these metabolites with other neurological or psychiatric conditions complicate their use as highly specific indicators for METH addiction. For instance, changes in amino acid metabolism and energy pathways are common to various neuropsychiatric disorders, potentially leading to false positives or negatives if used in isolation. Furthermore, the dynamic nature of metabolic profiles influenced by factors such as diet, circadian rhythms, and concurrent substance use can affect biomarker stability and detection sensitivity. Therefore, the development of reliable assays with high sensitivity to detect subtle metabolic shifts and high specificity to distinguish METH addiction from other conditions remains a critical hurdle for clinical translation.

4.3.2 Impact of individual variability on diagnostic accuracy

Individual differences pose another major challenge in the clinical implementation of biomarker-based diagnostics for METH addiction. Genetic background, age, sex, lifestyle factors, and the severity and duration of METH use can all influence the expression and regulation of exercise-mediated biomarkers. For example, the extent to which exercise normalizes disrupted metabolic pathways such as alanine, aspartate, and glutamate metabolism varies among individuals, potentially affecting the consistency and reproducibility of biomarker detection[135]. Additionally, comorbid conditions such as anxiety and cognitive impairments, which are prevalent in METH users, may independently alter metabolite levels like uric acid and tryptophan, confounding the interpretation of biomarker data[9]. These inter-individual variations necessitate personalized baseline assessments and adaptive diagnostic thresholds to enhance accuracy. Without accounting for such variability, biomarker-based diagnostics risk reduced predictive value and limited clinical utility.

4.3.3 Prospects for future multi-omics integrated diagnostic strategies

Looking forward, the integration of multi-omics approaches offers a promising avenue to overcome current limitations in sensitivity, specificity, and individual variability. This integration can build on the workflow for METH addiction biomarker research (Figure 3), using accessible samples (hair follicles, peripheral blood) to combine transcriptomic data including core biomarkers from the two-stage diagnostic model (Figure 4) with exercise-modulated metabolomic and epigenetic change[140]. Combining metabolomics with genomics, transcriptomics, proteomics, and epigenomics can provide a comprehensive molecular signature of METH addiction and its modulation by exercise. Such integrative strategies can identify robust biomarker panels that capture the complex pathophysiology of addiction and the systemic effects of exercise interventions. For instance, linking metabolic changes in neurotransmitter pathways with gene expression alterations in relevant brain regions may enhance diagnostic precision and reveal novel therapeutic targets. Moreover, advances in machine learning and bioinformatics can facilitate the analysis of high-dimensional multi-omics data, enabling personalized diagnostics tailored to individual molecular profiles. Although still in early stages, these integrated approaches hold great potential to revolutionize the diagnosis and treatment monitoring of METH addiction, ultimately improving clinical outcomes through precision medicine frameworks[9, 135]. Continued research and validation in diverse populations will be essential to translate these promising prospects into routine clinical practice.

5 The role of exercise-mediated biomarkers in methamphetamine addiction treatment

5.1 Exercise as an adjunct therapy: molecular basis

As an adjuvant treatment for methamphetamine addiction, the efficacy of exercise stems from its multi-level molecular effects on neural function and gene regulation, which is closely related to the regulation of biomarkers. At the molecular level, exercise induces a broad spectrum of physiological adaptations that enhance neural health and cognitive function. Acute physical activity triggers systemic molecular responses involving energy metabolism, oxidative stress modulation, inflammation attenuation, tissue repair, and growth factor signaling, as demonstrated by multi-omic profiling studies[150]. These molecular cascades contribute to improved neuronal plasticity and neurogenesis, partly mediated by upregulation of exercise-induced biomarkers such as BDNF, which plays a pivotal role in synaptic plasticity and cognitive enhancement[151]. The secretion of exerkines, which are key exercise-induced biomarkers including myokines, hepatokines, and adipokines, further orchestrates systemic and central nervous system adaptations, improving glucose metabolism, insulin sensitivity, and neuroprotection[64, 152].

The regulation of hallmark genes by exercise is tightly linked to behavioral improvements. For instance, exercise modulates gene expression profiles in skeletal muscle and brain tissues, influencing pathways related to mitochondrial function, inflammation, and cellular regeneration[153-154]. In neurodegenerative and addiction contexts, exercise-induced gene regulation can reverse or mitigate maladaptive molecular signatures, enhancing functional recovery[155-156]. Notably, exercise alters the expression of miRNAs, small non-coding RNAs that regulate gene networks implicated in addiction and neuroplasticity, some of which act as exercise-induced biomarkers, suggesting a mechanistic link between gene regulation and behavioral outcomes[157-158]. These molecular changes underpin improvements in cognitive function, mood regulation, and motor control, which are critical in addiction recovery and neurorehabilitation.

Moreover, exercise exerts synergistic effects when combined with pharmacological treatments. Exercise mimetics, which are pharmacological agents designed to replicate the molecular benefits of physical activity, target key signaling pathways such as AMPK, PGC-1α, and SIRT1, which are also modulated by exercise-induced biomarkers[159-160]. This synergy enhances therapeutic efficacy by promoting mitochondrial biogenesis, reducing oxidative stress, and modulating inflammatory responses. For example, in substance use disorders, exercise complements pharmacotherapy by reducing cravings, improving mood, and enhancing neuroplasticity, thereby supporting sustained recovery[19, 161]. Additionally, resistance and aerobic exercise have been shown to differentially influence neuroelectric dynamics and cognitive function in addicted individuals, suggesting tailored exercise interventions can potentiate drug treatment outcomes[156]. The integration of exercise into treatment regimens thus offers a holistic approach, addressing both molecular dysfunction and behavioral deficits.

In summary, the molecular basis of exercise as an adjunct therapy encompasses complex regulatory networks involving gene expression modulation, neurotrophic factor induction, and systemic signaling *via* exerkines. These molecular adaptations translate into behavioral improvements and potentiate pharmacological treatments, highlighting exercise's critical role in managing neuropsychiatric conditions, including addiction. Future research should focus on identifying specific molecular targets, optimizing exercise protocols, and exploring exercise-induced biomarkers to enhance the therapeutic potential of exercise in clinical practice and provide a scientific basis for new therapeutic strategies.

5.2 Gene-targeted exercise intervention strategies

Personalized exercise regimens designed based on individual gene expression profiles represent a promising frontier in precision medicine, particularly for complex disorders such as METH addiction. The rationale for tailoring exercise interventions to genetic characteristics stems from accumulating evidence that exercise modulates gene expression in a tissue and context-specific manner, influencing pathways relevant to addiction, metabolism, neuroplasticity, and inflammation. For instance, studies have demonstrated that exercise can alter the expression of microRNAs and transcription factors implicated in neural plasticity and addiction-related behaviors[78, 162]. In METH addiction models, exercise interventions have been shown to regulate gene networks involved in neurogenesis, synaptic function, and inflammatory responses, which are critical for cognitive recovery and relapse prevention[102, 129]. By integrating transcriptomic data from patients or animal models, exercise programs can be customized to target dysregulated genes or pathways, such as the GSK-3β/β-catenin pathway involved in hippocampal neurogenesis or the PTEN/AKT signaling axis implicated in cancer progression and potentially in

addiction neurobiology[129, 163]. This approach enhances the efficacy of exercise as a therapeutic modality by aligning the intervention with molecular signatures predictive of response, thereby maximizing neuroprotective and metabolic benefits while minimizing adverse effects.

The convergence of gene editing technologies with exercise interventions offers an innovative therapeutic paradigm that could synergistically correct pathological gene expression while harnessing the systemic benefits of physical activity. Gene editing tools such as CRISPR/Cas9 enable precise modulation of genes implicated in addiction and its comorbidities, including those governing neurotransmitter systems, neuroinflammation, and reward circuitry[164]. When combined with exercise, which itself induces beneficial epigenetic modifications and gene expression changes, this dual approach may potentiate recovery by both directly rectifying genetic abnormalities and promoting adaptive molecular remodeling. For example, exercise-induced upregulation of neurotrophic factors and modulation of RNA-binding proteins can complement gene editing effects to restore synaptic plasticity and cognitive function[78-79]. Moreover, gene editing could be employed to sensitize neural circuits to exercise-induced molecular cues or to enhance the expression of exercise-responsive genes, thereby optimizing treatment outcomes. Although this combined strategy remains largely exploratory, ongoing advances in delivery methods and safety profiles are paving the way for translational applications in addiction and other neurological disorders.

The development of exercise-mediated gene regulatory targets is critical for translating molecular insights into practical interventions for METH addiction and related conditions. Identification of key genes and pathways modulated by exercise provides a framework for biomarker discovery and therapeutic targeting, and some of these molecules can serve as Exercise-Induced biomarkers to monitor the regulatory effect of exercise on addiction-related gene pathways. For instance, exercise has been shown to influence the expression of genes such as NUP155, which regulates cell migration and invasion via the PTEN/AKT pathway, suggesting potential relevance beyond oncology into addiction neurobiology[163]. Similarly, genes involved in neuroinflammation (*e.g.*, IL-6, TNF-α), synaptic plasticity (*e.g.*, BDNF), and metabolic regulation (*e.g.*, PPARγ) are modulated by exercise and represent promising targets for intervention[111, 165-166]. High-throughput transcriptomic and metabolomic analyses have further identified gene sets and metabolic pathways altered by exercise in METH addiction models, including those related to amino acid metabolism, purine and pyrimidine metabolism, and glutamatergic signaling[9, 167], these exercise-modulated molecules (including gene products and metabolites) can act as exercise-induced biomarkers to provide the molecular basis for optimizing exercise protocols. These molecular targets can inform the design of exercise protocols optimized to activate beneficial gene networks. Additionally, understanding the epigenetic mechanisms by which exercise regulates gene expression, such as DNA methylation and histone modification, can facilitate the development of epigenetic biomarkers and guide the timing and intensity of exercise interventions[168-169]. Ultimately, the integration of molecular profiling with exercise physiology holds promise for advancing personalized medicine strategies that leverage gene-targeted exercise interventions to improve diagnosis, prognosis, and treatment of METH addiction and its sequelae.

5.3 Clinical implementation and effectiveness evaluation of exercise therapy

Optimizing the dose, frequency, and duration of exercise interventions is critical for maximizing therapeutic benefits across various clinical populations. Clinical trials have demonstrated that exercise therapy regimens ranging from moderate to high intensity, typically performed multiple times per week over several weeks to months, can significantly improve functional outcomes and quality of life. For example, in patients with knee osteoarthritis, supervised exercise programs conducted 1 – 2 times per week over 12 – 25 weeks have been shown to improve balance and physical function[170]. Similarly, in cancer survivors experiencing fatigue, exercise interventions with three sessions per week over 4 weeks demonstrated high adherence and feasibility, with trends toward reduced fatigue[171]. In cardiovascular rehabilitation, exercise therapy doses of 36 sessions resulted in substantial improvements in exercise capacity[172]. However, the optimal exercise dose is often individualized, dependent on patient tolerance, comorbidities, and specific clinical conditions. Notably, a systematic review on neck pain found that exercise therapy dosage parameters did not predict pain or disability outcomes, suggesting that exercise should be prescribed flexibly to accommodate patient needs[173]. Emerging technologies such as digital exercise therapy applications and wearable devices enable personalized exercise prescriptions and adherence monitoring, potentially enhancing dose optimization[174-175]. Overall, evidence supports tailoring exercise dose, frequency, and duration to individual patient profiles, with ongoing monitoring to adjust interventions for

maximal efficacy.

The integration of molecular biomarkers into the clinical monitoring of exercise therapy efficacy offers promising avenues for personalized treatment and outcome prediction. Studies have utilized biomarkers related to inflammation, neurogenesis, metabolic function, and stress responses to evaluate physiological adaptations to exercise interventions. For instance, in methamphetamine addiction, exercise-induced modulation of peripheral metabolites such as l-tryptophan and uric acid correlated with improvements in anxiety and cognitive function, suggesting their potential as biomarkers for treatment efficacy[9]. Similarly, exercise therapy in cancer patients has been associated with changes in endocrine markers including motilin and vasoactive intestinal peptide, which may reflect symptom improvement[176]. Near-infrared spectroscopy integrated within internet of medical things (IoMT) systems has been employed to monitor brain function changes after long-term exercise, demonstrating reductions in blood pressure and stress-related prefrontal cortex activity[177]. In chronic pain management, molecular and electrophysiological markers such as event-related potentials have been used to assess attentional bias changes following exercise in substance use disorders[178]. Additionally, transcriptomic analyses in animal models reveal exercise-induced gene expression changes in pathways related to neuroplasticity and inflammation, providing mechanistic insights[102].

However, translating these candidate biomarkers into routine clinical tools, such as blood-based tests, faces several practical challenges. These include pre-analytical and analytical variability, heterogeneity between assay platforms and patient populations, and the need to demonstrate added clinical value and cost-effectiveness compared with standard assessments[179]. Potential solutions include prioritizing robust biomarkers that can be reliably measured in easily accessible specimens (*e. g.*, plasma), developing standardized and quality-controlled assays, validating biomarker panels across independent cohorts, and embedding biomarker testing into prospective clinical trials that formally evaluate clinical utility and health-economic impact[180]. These findings underscore the potential of biomarker-driven monitoring to objectively assess and optimize exercise therapy, facilitating precision rehabilitation strategies.

Exercise therapy exhibits variable applications and effects across different stages of addiction, from initiation and active use to withdrawal and long-term recovery. During early withdrawal, exercise can alleviate withdrawal symptoms, reduce cravings, and improve mood and cognitive function, as evidenced in opioid and methamphetamine use disorders[181-182]. For example, aerobic exercise has been shown to diminish attentional bias to drug-related cues and modulate neurophysiological correlates of addiction in methamphetamine users[178]. In maintenance phases, exercise supports neurobiological recovery by enhancing neurogenesis and modulating neurotransmitter systems implicated in addiction[129, 182]. Moreover, exercise interventions during rehabilitation can improve physical health comorbidities, reduce relapse risk, and enhance quality of life[161, 183]. Social and psychological factors also influence exercise engagement; for instance, patients receiving opioid agonist therapy report preferences for group exercises but express concerns about exposure to substance-using peers, highlighting the need for tailored program designs[184]. Additionally, the intensity and type of exercise may differentially impact addiction outcomes, with moderate to vigorous aerobic exercise showing benefits in psychological symptoms and craving reduction[183]. Overall, exercise therapy should be adapted to the stage of addiction, patient preferences, and clinical context to maximize therapeutic efficacy and support sustained recovery.

5.4 Combined strategy of exercise intervention and gene editing

Exercise intervention, as a non-pharmacological therapeutic approach, exhibits considerable potential in regulating gene expression and restoring physiological dysfunction. Integrating transcriptome data analysis from patients and animal models enables the precise identification of key dysregulated genes and pathways associated with methamphetamine addiction, thereby providing a scientific basis for the design of individualized exercise regimens. Transcriptome technology facilitates the revelation of abnormal states of multi-level mechanisms including intracellular signal transduction, metabolic regulation, and inflammatory responses in nerve cells during the addiction process by comprehensively depicting changes in gene expression profiles[185]. For instance, exercise training has been demonstrated to regulate the expression of genes related to mitochondrial function, oxidative stress, and neuroprotection, improve the energy metabolism and anti-inflammatory capacity of relevant cells, and thereby alleviate the symptoms of metabolic and neurodegenerative diseases[185-186].

In animal models, exercise intervention regimens optimized based on transcriptome data can target specific dysregulated pathways, such as by enhancing the autophagic mechanism of cardiomyocytes or regulating the signal network of the central nervous system, thus improving the therapeutic effect of

exercise[185]. Furthermore, the application of gene editing technologies such as CRISPR/Cas9 provides a means to correct gene defects under pathological conditions. CRISPR technology enables precise knockout, knock-in, or regulation of specific genes, and when combined with exercise-induced changes in gene expression, it forms a synergistic effect to maximize therapeutic outcomes[187-188]. For example, combining exercise training to promote the activation of neuroprotective signaling pathways with the application of gene editing to repair or regulate relevant pathological genes is expected to achieve dual intervention in addiction-related nerve damage.

The combined application of gene editing and exercise intervention can not only target single therapeutic sites but also contribute to regulating complex gene networks and signaling pathways, thereby achieving systematic repair. This strategy is particularly suitable for the multi-dimensional pathological characteristics of methamphetamine addiction, where neuroinflammation, metabolic imbalance, and changes in neural plasticity are intertwined. In-depth mining of transcriptome data allows for the precise identification of gene expression differences at various stages and among different individuals, providing a basis for formulating personalized exercise prescriptions and selecting gene editing targets, which in turn improves therapeutic efficacy and safety[189-190].

Currently, the role of exercise intervention in improving skeletal muscle function, delaying aging, and exerting neuroprotective effects has been widely confirmed, and its impact on epigenetic modifications provides the possibility of synergistic regulation with gene editing technologies[191]. Meanwhile, verifying the efficacy of the combined intervention of gene editing and exercise using animal models helps to reveal its molecular mechanisms, optimize therapeutic regimens, and promote clinical translation. Future research should focus on multi-omics data integration, explore the synergistic network between exercise-induced gene regulation and the effects of gene editing, develop precise and personalized therapeutic strategies, and ultimately achieve effective intervention and functional recovery in methamphetamine addiction[187-188].

6 Conclusion and prospect

The intricate interplay between exercise and METH addiction at the molecular level represents a pivotal frontier in addiction neuroscience and therapeutic intervention. This review underscores that exercise exerts profound modulatory effects on the neurobiological substrates of METH addiction by regulating specific biomarkers, which act as critical nodes in complex regulatory networks governing neurotransmitter systems, neuroinflammation, neuroplasticity, and epigenetic modifications. These multifaceted molecular mechanisms not only deepen our understanding of METH addiction pathophysiology. They also illuminate novel avenues for diagnosis and treatment, with biomarkers serving dual roles as potential biomarkers for early detection of addiction progression and as mechanistic targets for therapeutic intervention. Notably, exercise intervention exhibits prominent complementary effects with existing pharmacotherapies for drug addiction[192]. Current medications, such as dopamine receptor modulators, primarily alleviate acute withdrawal symptoms but are often accompanied by adverse effects including nausea and insomnia, and fail to effectively reduce long-term relapse rates[184]. In contrast, exercise modulates neuroplasticity and neuroinflammatory pathways that are less targeted by conventional drugs, thereby enhancing the therapeutic efficacy of medications, mitigating their side effects, and improving long-term prognosis[193]. This synergistic interaction positions exercise as a crucial component of comprehensive addiction management strategies. However, the heterogeneity in study designs, participant populations, and exercise protocols across existing research necessitates cautious interpretation of findings. It also highlights the urgent need for standardized methodologies to harmonize results and enhance the reproducibility of exercise-mediated biomarkers regulation studies.

To address these challenges and advance mechanistic understanding, multi-omics integrative analysis, which spans genomics, transcriptomics, and metabolomics, holds substantial promise for research on exercise-mediated biomarkers regulation and METH addiction. This integration enables a systems-level perspective, unraveling how genetic variants, gene expression changes, and metabolic alterations collectively drive exercise-induced physiological adaptations and modulate addiction-related pathological states. This approach offers an advantage that surpasses insights from single-omics studies. For instance, large-scale multi-omics analysis in human skeletal muscle has identified distinct molecular signatures linked to exercise modalities[194], while similar approaches in disease contexts have uncovered novel biomarkers by correlating DNA methylation, RNA expression, and metabolite profiles[195-196]. The advent of big data and artificial intelligence (AI) further amplifies this potential. AI-driven bioinformatics tools facilitate the processing of heterogeneous omics data, as demonstrated in precision oncology for biomarker identification and patient stratification[197], while digitalomics

technologies accelerate data interpretation and improve the accuracy of clinical insights[198]. Specifically, these models leverage multi-omics data integration, for instance by identifying biomarkers such as tumor mutational burden and immune cell infiltration patterns in cancer immunotherapy[199], or by optimizing diagnostic and prognostic prediction protocols through transcriptomic and clinical datasets in ulcerative colitis research[200]. Furthermore, in miRNA-related cancer studies, machine learning algorithms have been applied to identify key biomarkers and construct prognostic models, which further demonstrates the potential of AI in integrating high-throughput data to accelerate biomarker discovery[201]. Additionally, frameworks like Bioconductor packages, spatial multiomics platforms, and single-cell multiomics technologies address technical challenges such as batch effects and cellular heterogeneity. These advances lay the groundwork for translating complex molecular data into clinically actionable insights[202-203]. Continued development of computational tools and standardized data integration pipelines will be critical to fully harnessing multi-omics for METH addiction research.

Building on these technological advances, elucidating the nuanced molecular mechanisms underlying exercise interventions remains a key priority for optimizing therapeutic strategies against METH addiction. Exercise-induced biomarkers regulation is highly dependent on cell type specificity. In skeletal muscle, exercise stimulates mitochondrial biogenesis and lipid metabolism via pathways involving PGC-1α and SIRT1, as shown in aged obese female mice where hepatic Sirt1 mRNA upregulation correlated with improved fatty acid oxidation[204]. In the brain, treadmill exercise altered hippocampal transcriptomes in METH-addicted mice, regulating genes related to immune responses and neuroplasticity to promote recovery[8, 102], and tissue-specific responses to exercise also modulate neuroendocrine-immune biomarkers[205]. Furthermore, exercise modality and intensity shape gene expression profiles. Endurance exercise enhances mitochondrial function in skeletal muscle, while strength training prioritizes protein synthesis[206]. In METH addiction models, moderate-intensity aerobic exercise reduced inflammatory cytokines and improved cognitive function[207], whereas high-intensity exercise acutely elevated neuroendocrine markers[205], with microRNAs implicated in muscle atrophy and neuroplasticity also showing modality-specific regulation[208]. Long-term exercise induces sustained adaptations, such as preserved hippocampal volume in older adults[209], reduced attentional bias toward drug cues in METH users[178], and modulation of signaling pathways[102]. However, some molecular markers may not change despite functional improvements[210]. Dissecting these mechanisms will enable more precise exercise prescriptions.

Despite growing clarity on these mechanisms, translating research findings on METH use disorder (MUD) from bench to bedside faces significant challenges that hinder clinical application. A primary barrier is the heterogeneity of METH pathology, driven by interactions between genetic backgrounds and environmental factors[210], which complicate the establishment of universal diagnostic thresholds for biomarkers like circulating miRNAs and inflammatory ratios[9, 210]. Standardization and scalability of biomarker assays also pose issues. Advanced technologies are resource-intensive and not widely accessible in routine clinical settings, necessitating the development of simplified, cost-effective assays. Additionally, implementing personalized exercise interventions requires addressing practical hurdles, including patient adherence, variability in exercise capacity, and the need to tailor programs to individual factors[140]. This process demands multidisciplinary collaboration among clinicians, researchers, and rehabilitation specialists. Furthermore, age and gender factors significantly exacerbate interindividual differences in exercise responsiveness[211], for instance, compared with male patients, female patients exhibit distinct gene expression profiles during aerobic exercise, which may be attributed to gender-specific hormonal regulation and genetic polymorphisms[212]. Safety considerations also constitute a crucial barrier, especially for patients with cardiovascular dysfunction or comorbid mental illnesses, who require personalized pre-exercise health assessments[213]. However, such assessments are typically resource-intensive and insufficiently implemented in clinical practice[213-214]. Additionally, the shortage of professional medical personnel proficient in both addiction medicine and exercise physiology, coupled with the uneven geographical distribution of rehabilitation resources, severely limits the generalizability and practical application effects of evidence-based exercise interventions, particularly in low- and middle-income regions[214]. Longitudinal studies are further needed to assess biomarker stability over the course of MUD treatment and recovery[215-217].

In summary, exercise-mediated regulation of biomarkers represents a transformative approach to preventing and treating METH addiction. It bridges molecular neuroscience with clinical practice to shift addiction management from symptomatic treatment to targeted, mechanism-based interventions. The

integration of multi-omics technologies with AI-driven analytics offers unprecedented opportunities to unravel complex molecular networks, while in-depth exploration of exercise's cell type-specific, modality-dependent, and long-term effects will refine intervention strategies. Overcoming clinical translation challenges through rigorous validation of biomarkers, development of accessible diagnostic tools, and implementation of personalized protocols will require sustained interdisciplinary effort, advanced bioinformatics support, and well-designed clinical trials. By addressing these priorities, the field can fully harness the therapeutic potential of exercise, ultimately improving diagnostic accuracy, monitoring treatment response, and enhancing patient outcomes in methamphetamine use disorder.

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运动诱导甲基苯丙胺成瘾的生物标志物：分子机制和临床意义

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摘要　甲基苯丙胺（METH）成瘾是一种严重的神经精神疾病，现有诊断与治疗手段仍然有限。运动作为安全、可及的非药物干预，其改善METH成瘾的作用与一系列运动诱导生物标志物的动态调控密切相关。本综述在概述METH所致神经递质失衡、神经炎症、氧化应激及表观遗传异常等分子病理基础上，重点阐述运动通过调节BDNF、炎症因子、miRNAs等关键标志物，进而影响多巴胺、谷氨酸等神经递质系统、神经炎症以及神经可塑性，从而部分逆转METH诱导的神经生物学紊乱并改善成瘾相关行为。同时，本文讨论了利用外周血等易获取组织的转录组、代谢组和免疫相关基因表达谱，构建多标志物模型并动态监测运动应答，用于METH成瘾早期识别、疗效评估及人群分层的可行性和应用前景，旨在为运动介导生物标志物在METH成瘾精准诊疗与个体化干预中的转化应用提供理论依据。

关键词　甲基苯丙胺成瘾，运动干预，生物标志物，诊断，治疗

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