Review

MOLECULAR METABOLISM

Three-dimensional network of creatine metabolism: From intracellular energy shuttle to systemic metabolic regulatory switch

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ABSTRACT

Background: Creatine serves as an intracellular shuttle for high-energy phosphate bonds, enabling rapid ATP transfer from energy-producing to energy-consuming cellular compartments. In skeletal muscle, creatine coordinates energy distribution among mitochondrial oxidative phosphorylation, glycolysis, and the phosphagen system. Consequently, creatine supplementation acutely enhances muscular performance and is widely utilized as an ergogenic aid in power-based sports. Recent studies demonstrate that enhanced creatine metabolism in adipose tissue promotes brown adipocyte renewal and boosts energy expenditure in cold environments or sedentary conditions, thereby improving overall systemic metabolism. Beyond its traditional role as an exercise supplement, the creatine metabolic network has emerged as a promising therapeutic target for metabolic disorders.

Scope of review: This review begins by revisiting the history and latest advancements in creatine research, and ultimately proposes three dimensions for the current explanation of creatine metabolism: (1) subcellular energy transport; (2) muscle-fat metabolic axis; (3) systemic energy sensing and metabolic reprogramming.

Major conclusions: The creatine cycle enables directed energy flow through mitochondrial supercomplexes (VDAC/ANT-CK) and resets systemic metabolism via subcellular energy tunnels and inter-organ interactions. Creatine kinase (CK) condensates, through liquid—liquid phase separation, can rapidly meet energy demands during exercise. Therefore, targeting the dynamics of the CK phase may be promising for enhancing athletic performance and improving metabolic diseases.

Keywords Creatine; Exercise; Energy metabolism; Metabolic diseases; Thermogenesis; Phase separation

1. INTRODUCTION

Creatine (methylguanidinoacetic acid), first isolated from skeletal muscle by French chemist Michel Eugène Chevreul in 1832, has undergone nearly two centuries of functional exploration. For much of the 20th century, research on this quintessential skeletal muscle metabolite remained confined to exercise bioenergetics. The phosphocreatine (PCr) shuttle, sustaining ATP homeostasis via creatine kinase (CK)-catalyzed reversible reactions, became the canonical framework explaining energy provision during high-intensity, short-duration exercise. However, emerging evidence from metabolomics, single-cell sequencing, and CRISPR-based gene editing has unveiled an expansive creatine metabolic network across adipose tissue, liver, and the central nervous system. This network orchestrates systemic metabolic homeostasis through modulation of cellular energy sensors (AMPK/mTOR) and mitochondrial dynamics [1]. Landmark studies delineating the creatine-adipose thermogenic axis mark a paradigm shift in our understanding, from viewing creatine merely as an "ergogenic aid" to recognizing its role as a "metabolic rheostat" [2—5]. This conceptual evolution not only redefines creatine biological significance but also opens new therapeutic avenues for metabolic disorders.

2. THE THREE PARADIGM SHIFTS IN CREATINE METABOLISM

2.1. The energy buffer hypothesis (1832—1980)

The biological investigation of creatine originated in 1832 with its isolation from skeletal muscle as a nitrogenous organic compound, named "creatine" (from Greek *kreas*, meaning flesh). Early studies, constrained by analytical limitations, focused solely on its tissue distribution until the discovery of phosphocreatine (PCr) in the early 20th century unveiled its metabolic role. In 1927, identification of PCr as a high-energy phosphate compound in muscle extracts prompted hypotheses about its involvement in ATP resynthesis [6], catalyzing systematic exploration of creatine metabolism. A pivotal breakthrough occurred in 1933 with the characterization of creatine kinase (CK), which mediates the reversible phosphorylation between creatine and ATP ($Cr + ATP \leftrightarrow PCr + ADP$), establishing the enzymatic foundation of the energy buffer theory [7].

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Radioisotope tracer studies in the 1950s revealed dynamic PCr homeostasis in skeletal muscle. At rest, intramuscular PCr concentrations reach 20—35 mM (4- to 5-fold higher than ATP) forming an expansive high-energy phosphate reservoir. During intense contraction, PCr levels plummet by 30—50%, while ATP fluctuates minimally (∼10%), demonstrating PCr's role as the primary defense for ATP homeostasis. These observations crystallized the energy buffer hypothesis: PCr buffers abrupt ATP/ADP ratio shifts via CK-mediated phosphate transfer, sustaining energy-demanding processes like myosin ATPase activity [8]. In 1966, Bessman proposed the "phosphocreatine shuttle" concept, suggesting compartmentalized CK isoforms (e. g. , mitochondrial CK vs. cytosolic CK) create energy relay pathways [9]. However, technological limitations precluded structural validation.

Electron microscopy in the 1970s provided critical morphological evidence. Mitochondrial CK (CK-MT) densely localizes to cristae membranes, forming nanoscale complexes with adenine nucleotide translocase (ANT), while cytosolic CK-MM anchors at myofibrillar Z-discs, functionally coupling with sarcoplasmic reticulum Ca^{2+}-ATPases. This spatial organization implies a compartmentalized energy circuit. Mitochondrial ATP is rapidly converted to PCr via CK-MT, diffuses cytosolically, and regenerates ATP at MM-CK sites, establishing an "ATP-PCr-ATP" energy buffer loop [10,11]. CK-MT exhibits clear tissue specificity, primarily expressed in cardiac muscle and skeletal muscle, and is not expressed in the liver, ensuring that the heart and muscles can rapidly obtain energy. Perfusion experiments in 1980 demonstrated PCr-mediated ATP regeneration operates ∼3 orders of magnitude faster than oxidative phosphorylation, elegantly explaining energy provision during instantaneous high-intensity efforts like sprinting [12].

The classical "CK-PCr" model thus emerged with three points: PCr as a mobile phosphate carrier overcoming ATP diffusion constraints; Compartmentalized CK isoforms enabling directional energy transfer; ATP/PCr buffering maintaining cellular energy homeostasis. This framework not only shaped exercise bioenergetics but also revolutionized understanding of energy dysregulation in myocardial ischemia and neurodegenerative diseases. While subsequent research revealed broader metabolic regulatory roles of creatine, the energy buffer hypothesis remains foundational for deciphering intracellular energy transduction.

2.2. The energy shuttle theory (1981—2015)

Breakthroughs in molecular biology during the 1980s propelled the transition from static buffering models to dynamic shuttling paradigms in creatine research. In 1981, Bessman formalized the "phosphocreatine shuttle", positing that compartmentalized CK isoforms constitute the molecular basis for energy channeling [13]. In 1984, Meyer, R. A quantitatively analyzed the phosphocreatine shuttle, concluding that for cells with small distances between ATP utilization and generation sites, such as cardiac myofibrils and mitochondria, the phosphocreatine shuttle is not significant [14]. A study from 1985 further showed that there is a big difference in phosphometabolites between fast and slow muscle fibers in mammals [15]. Immunofluorescence and subcellular fractionation in the 1990s confirmed spatial specialization. 75% of skeletal muscle CK-MT anchors to mitochondrial inner membranes, forming fixed stoichiometric complexes with ANT (1:1.2), while cytosolic MM-CK (muscle-type) enriches at myofibrillar M-bands, colocalizing with myosin heavy chains. This spatial stratification revealed a trans-compartmental energy highway: mitochondrial ATP is converted to PCr via CK-MT, diffuses along concentration gradients to cytosolic sites, and regenerates ATP at energy-demanding loci through MM-CK catalysis [16].

In 1994, Saks quantified the efficiency of compartmentalized energy transfer within cells using isolated mitochondria. The co-localization of CK-MT/ANT increased the rate of PCr synthesis, significantly reducing the ADP diffusion delay compared to a randomly distributed system [17]. This thermodynamically validated the energy shuttle hypothesis. CK isoenzymes physically couple the sites of ATP generation and consumption, replacing the diffusion-dependent random collision mechanism with a directed metabolic tunnel. By 1998, confocal microscopy successfully captured the concentration gradient of PCr around mitochondria during type II fiber contraction, directly providing power for ATP regeneration in the Z-disk region [18]. However, studies in the 1990s suggested that Cr/PCr did not stimulate mitochondrial respiration *in vitro* [19], which became the fundamental argument for Saks et al.'s shift to permeabilized fibres. By re-evaluating Walsh's study published in The Journal of Physiology in 2001 [20], we found that the higher concentration of mitochondrial suspension used previously (10 mg/mL, heart mitochondria) might have masked the regulatory effect of Cr/PCr [21]. This contrasts sharply with later experiments using low concentrations of mitochondria (0.5 mg/mL) combined with permeabilized fibres [22,23]. Excessively high mitochondrial concentrations may exceed the regulatory range of Cr/PCr, which explains why previous studies did not find that Cr/PCr regulates mitochondrial respiration. Currently, it has generally been believed that the CK-creatine system may exist as a secondary regulatory mechanism for mitochondrial respiration [24].

Post-2000 super-resolution microscopy further corroborated intracellular compartmentalization for energy metabolism. CK-MT crystallography identified dual anchoring, an N-terminal mitochondrial targeting sequence (31 residues) and a C-terminal cardiolipin-binding motif, ensuring stable integration at mitochondrial contact sites [25]. CK-MT knockout impaired skeletal muscle peak power output and delayed PCr resynthesis, whereas MM-CK ablation reduced sarcoplasmic reticulum Ca^{2+} reuptake efficiency, confirming isoform-specific energy allocation roles [26]. However, Dzeja et al. have shown that inhibiting CK alone does not acutely limit left ventricular function in normal hearts. When an important pathway in the bioenergetic network is removed (such as the CK system), support systems such as adenylate kinase (AK), glycolysis, and guanine nucleotide phosphate transfer pathways are activated to meet the high-energy phosphate demand at the end of the contractile apparatus. The rearrangement of the phosphotransfer and substrate utilization networks provides compensation for CK deficiency [27]. The progress during this period more comprehensively defined the intracellular energy paradigm, with the 'dual-mode' of PCr shuttle-local regeneration and ATP diffusion providing better metabolic resilience for tissue and cells. Kazak revealed that adipocyte CK-MT drives proton leak through PCr hydrolysis, achieving 60% of UCP1-mediated thermogenic capacity and extending the energy shuttle theory beyond muscle to adipose systems [28].

2.3. Systemic regulatory paradigm (2016—Present)

Since 2016, advancements in single-cell sequencing and metabolomics have led to a systematic transformation in creatine biology. Exogenous creatine supplementation effectively activates AMPK signaling in adipose tissue (phosphorylation↑) and induces browning of white fat (UCP1 expression↑). This process reveals the crucial role of creatine in systemic energy balance through metabolic crosstalk between muscle and fat tissues [29]. This discovery transcends traditional exercise bioenergetics, establishing creatine as a systemic

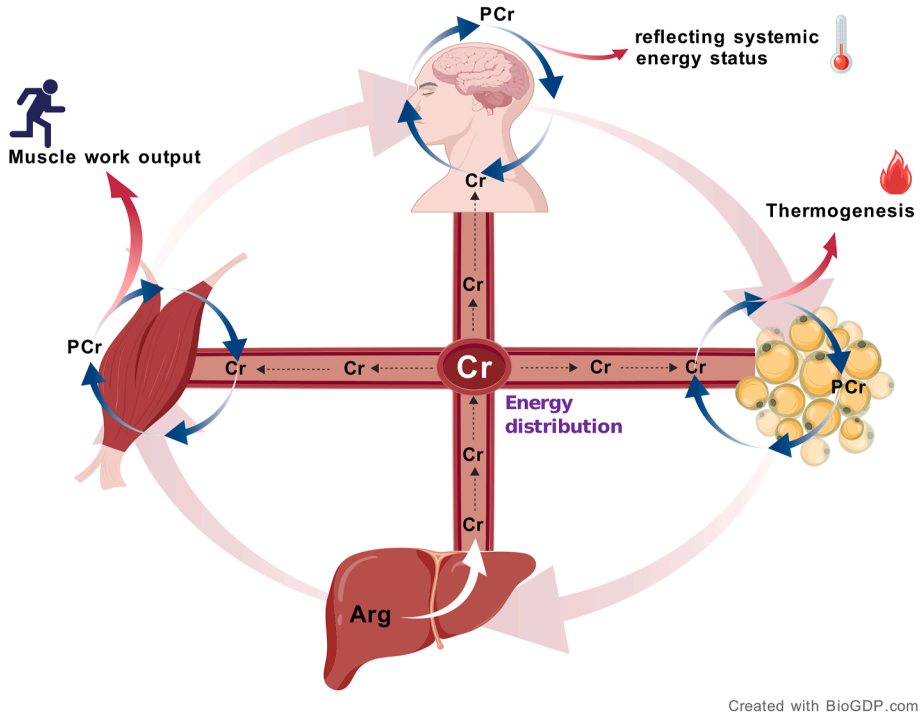

Figure 1: The BLSA Metabolic Axis Model of Creatine. Creatine synthesized by the liver is distributed to various tissues and organs through the bloodstream, entering the intracellular creatine cycle. The creatine cycle in skeletal muscle is coupled with ATP synthesis, consuming energy in the form of work output (exercise); the creatine cycle in adipose tissue is coupled with thermogenesis, consuming energy in the form of heat. The creatine cycle in the brain can sense the system energy metabolic status and subsequently regulate the energy expenditure relationship between tissues and organs through neural and endocrine mechanisms. This model defines creatine as the core regulator of systemic energy distribution, enabling diverse energy allocation through creatine cycles in different tissues. This model not only reveals how creatine coordinates energy distribution among different tissues but also suggests that regulating creatine metabolism could be a new strategy for treating metabolic diseases. By controlling creatine cycle, it may be possible to achieve fine-tuned management of energy metabolism, thereby reversing pathological processes of obesity, diabetes, and other metabolic diseases.

metabolic regulator. Creatine precisely coordinates energy sensing through dual pathways of AMPK and mTOR: when energy supply is limited, AMPK activates catabolism; whereas, when energy supply is abundant, mTOR promotes cellular anabolism. When energy supply is tight, PCr depletion increases the AMP/ATP ratio, directly activating AMPK. Creatine binds to the Bateman domain of the AMPK gamma subunit, causing conformational changes that enhance catalytic activity [30]. Acute creatine exposure activates mTORC1 via PI3K/Akt (S6K phosphorylation↑), while long-term supplementation upregulates Sestrin2 to inhibit mTORC1, establishing a balance between anabolism and catabolism [31]. This biphasic regulation explains the insulin-sensitizing effect of creatine. Mice on a high-fat diet showed increased glucose uptake in skeletal muscle and reduced lipid deposition in the liver after supplementation with creatine [32,33].

Mitochondrial dynamics studies further expand creatine's regulatory scope. Cryo-electron microscopy (Cryo-EM) structural analyses demonstrate creatine stabilizes the ANT "C-state" conformation by binding its Glu^{264} residue, boosting ADP/ATP exchange efficiency [34]. Concurrently, creatine upregulates fusion protein Opa1 and inhibits fission factor Drp1, promoting mitochondrial networking and oxidative phosphorylation capacity [35]. This structural-functional rewiring is pronounced in adipose tissue. UCP1-knockout mice achieve 72% of wild-type thermogenic efficiency via CK-MT-mediated futile cycling, coupled with reduced mitochondrial ROS production [36]. This may be because CK-MT exerted antioxidant effects, reducing mitochondrial ROS production through the ADP cycle mechanism [37]. Creatine mediates cross-talk between adipocytes and cancer cells, regulating obesity-driven breast cancer. This finding reveals that the key node of communication between adipocytes and cancer cells lies in the direction of creatine [38]. High BMI is associated with lower hypothalamic N-acetylaspartate (NAA)/Cr ratios, suggesting extensive interactions among hormonal imbalances, neurohormonal changes, and hypothalamic function in obese adolescents [39]. These studies further suggest that creatine, as a crucial energy carrier, facilitates crosstalk among many tissues and organs, with the allocation of creatine resources serving as a mode of communication between them. Thus, creatine has evolved from a phosphate shuttle to a *systemic metabolic orchestrator*, integrating energy sensing (AMPK/mTOR), allocation (mitochondrial networking), and dissipation (futile cycling).

3. THE BLSA METABOLIC AXIS OF CREATINE

Recent studies have redefined creatine's biological role, shifting from its function as a skeletal muscle energy buffer to a key player in systemic metabolic regulation. Multi-organ metabolomics and single-cell sequencing highlight creatine's crucial role in the "brain (regulation)—liver (synthesis)—skeletal muscle (energy flux)—adipose (dissipation)" metabolic axis (BLSA metabolic axis, Figure 1). This model demonstrates how spatiotemporally coupled creatine networks manage systemic energy distribution, providing new insights for integrated metabolic disease interventions [40].

3.1. The brain as the command center of creatine networks

Hypothalamic arcuate nucleus neurons express high-density creatine transporters (SLC6A8), with intracellular PCr/ATP dynamics reflecting systemic energy status. Fasting-induced PCr/ATP depletion activates AgRP neurons to stimulate feeding, while exogenous creatine infusion suppresses NPY expression via AMPKα2 activation, reducing food intake [41]. Mechanistically, brain-derived neurotrophic factor (BDNF) upregulates adipose CK-MT expression, establishing long-range brain-adipose axis crosstalk [42]. Clinical neuroimaging reveals significantly lower prefrontal cortical PCr levels in obese patients versus controls, inversely correlated with HOMA-IR indices, implicating cerebral energy dysregulation as a driver of axis imbalance [43]. Creatine supplementation increases cerebral creatine reserves, potentially explaining its neurocognitive benefits in aging and metabolic stress (e.g., sleep deprivation). Emerging evidence further highlights creatine's therapeutic potential in mitigating outcomes of muscular dystrophy, traumatic brain injury (including pediatric concussion), and mood disorders [44,45]. In a mouse model deficient in ornithine acetyltransferase (GAMT$-/-$) leading to creatine (Cr) deficiency, researchers investigated changes in creatine levels in skeletal muscle and brain after creatine supplementation. The results showed that creatine levels in skeletal muscle increased faster than in the brain, but this phenomenon only occurred on the first day of supplementation. Subsequently, creatine levels in all studied areas increased at a rate of 0.8 mM per day [46]. This study also revealed a competitive relationship between skeletal muscle and brain in creatine uptake, which actually reflects the interaction mechanism between brain and muscle.

3.2. The liver as the biosynthetic hub

The liver serves as the primary site for endogenous creatine synthesis, maintaining systemic creatine homeostasis through enzymatic cascades. In the canonical pathway, arginine—glycine amidinotransferase (GATM) catalyzes guanidinoacetate production in the kidneys, which is subsequently methylated by guanidinoacetate N-methyltransferase (GAMT) in the liver to form creatine. Crucially, insulin/IGF-1 signaling upregulates GAMT activity, while AMPK activation suppresses the methyl donor function of S-adenosylmethionine (SAM), establishing a *synthesis-catabolism equilibrium* [47]. Clinical studies demonstrate reduced hepatic GAMT activity in nonalcoholic fatty liver disease (NAFLD) patients, concomitant with decreased plasma creatine and skeletal muscle PCr reserves, implicating hepatic dysfunction in systemic metabolic dysregulation [48]. Notably, creatine or guanidinoacetate supplementation attenuates hepatic lipid accumulation in NAFLD murine models, solidifying the liver's role as a *metabolic gatekeeper* within the quadruple axis [49,50].

3.3. Skeletal muscle: the powerhouse and metabolic sentinel

Accounting for $>$95% of total body creatine stores, skeletal muscle forms the structural and functional cornerstone of the metabolic axis. At the subcellular level, creatine enables directional energy flux via the PCr shuttle system, enhancing energy transfer efficiency. Cryo-EM structural analyses reveal that VDAC/ANT-CK supercomplexes form nanochannels under membrane potentials $>$150 mV, tripling PCr synthesis rates compared to free diffusion [51,52].

Cytosolic ADP transport to mitochondria, a key regulator of metabolic homeostasis, is critically modulated by the PCr/Cr shuttle. Surprisingly, mitochondrial CK knockout (disrupting VDAC/ANT-CK complexes) does not impair exercise tolerance in mice, indicating redundant mechanisms for energy balance maintenance during activity. However, KO mice exhibit a 50% reduction in the Km for mitochondrial ADP sensitivity post-exercise, demonstrating VDAC/ANT-mediated ADP transport operates independently of PCr/Cr cycling [53]. Beyond energy production, skeletal muscle functions as a metabolic sentinel. Exercise-induced mechanical stress activates the AMPK/mTOR axis via CK/PCr dynamics, triggering PGC-1α-mediated mitochondrial biogenesis. Concurrently, myokine secretion (e. g. , IL-6, irisin) initiates adipose tissue browning and hepatic gluconeogenic reprogramming [32].

3.4. Adipose tissue as the thermogenic effector

Emerging insights into adipose creatine metabolism have revolutionized classical thermogenic paradigms. Brown adipocyte mitochondrial CK drives proton leak through PCr hydrolysis, enabling UCP1-independent heat production. This mechanism is particularly prominent in UCP1-knockout models, cold exposure upregulates adipocyte creatine transporter *SLC6A8* via β3-adrenergic receptor-PKA signaling, activating futile creatine cycling to convert energy into heat with 78% efficiency [54]. Crucially, adipose-specific knockout of the rate-limiting enzyme *Gatm* reduces basal metabolic rate and exacerbates diet-induced obesity, confirming creatine metabolism as a master regulator of adipocyte thermogenesis [29]. Metabolic tracing further reveals aged inguinal white adipose tissue (iWAT) actively exports creatine to skeletal muscle, compensating for brown adipose dysfunction, a striking interorgan energy redistribution mechanism underpinning the *adipose-muscle metabolic axis* [55].

The creatine metabolic network functions through spatiotemporal synergy in three dimensions. (1) Spatial precision: VDAC/ANT-CK supercomplexes facilitate subcellular energy flow through engineered pathways. (2) Temporal adaptation: AMPK/mTOR signaling pathways interpret real-time PCr changes to trigger context-specific metabolic adjustments. (3) Systemic integration: Brain-liver-muscle-adipose interorgan feedback loops exchange creatine metabolites for overall energy regulation.

4. THE EVOLUTIONARY ADVANTAGE OF FUTILE CYCLING

The evolution of mammalian thermogenic mechanisms reflects an optimization between energy conversion efficiency and oxidative stress costs. While brown adipose tissue (BAT) traditionally relies on UCP1-mediated proton leak for non-shivering thermogenesis (NST), this process incurs substantial reactive oxygen species (ROS) production. Emerging evidence reveals that creatine-driven futile cycling achieves comparable thermogenic output with markedly reduced ROS generation, a low-cost adaptation explaining how arctic mammals maintain body temperature despite minimal UCP1 expression [56].

4.1. Metabolic cost disparity: UCP1 vs. creatine cycling

UCP1 uncouples oxidative phosphorylation by dissipating proton gradients, directly converting chemical energy to heat. Per glucose molecule consumed, UCP1 generates 5.6 kcal heat but reduces ATP synthesis efficiency by 72% [57]. Critically, electron transport chain (ETC) uncoupling exacerbates electron leakage. At $-$80 mV membrane potential, UCP1 activation elevates superoxide ($O_2^{\cdot-}$) production, with 40% originating from Complex I's FMN moiety. This oxidative burden forces compensatory upregulation of superoxide dismutase (SOD2) and glutathione peroxidase (GPX), depleting NADPH reserves to maintain redox balance [58].

In contrast, creatine cycling dissipates energy via phosphorylation-dephosphorylation. CK-MT phosphorylates creatine using ATP, while tissue-nonspecific alkaline phosphatase (TNAP) hydrolyzes PCr to release inorganic phosphate (Pi) and heat. Each creatine cycle

consumes 1 ATP equivalent ($\Delta G'^{\circ} = -10.3$ kcal/mol) yet yields 9.1 kcal heat, an 88% thermodynamic efficiency [57]. In brown adipocyte, CK condensates colocalize TNAP, achieving 65% of UCP1-mediated thermogenic efficiency [59]. Strikingly, mitochondrial-targeted CKB reintroduction rescues cold tolerance in UCP1/CKB double-KO mice via TNAP-dependent creatine cycling, establishing this pathway as a bona fide thermogenic mechanism [60]. Mechanistically, PCr synthesis stabilizes ANT-VDAC supercomplexes, enhancing ETC Complex I/III/IV alignment to minimize electron leakage. CK-MT physical coupling with F_1F_0-ATP synthase accelerates ADP recycling, stabilizing ETC flux [61].

Comparative studies of arctic foxes (*Vulpes lagopus*) and red foxes (*Vulpes vulpes*) demonstrate this evolutionary optimization. Arctic fox interscapular fat exhibits 6. 8-fold higher creatine cycling activity but only 23% UCP1 protein levels versus red foxes. Despite -30 °C exposure, arctic foxes maintain body temperature with 41% lower ROS production than UCP1-dependent red foxes [62]. These findings establish the evolutionary supremacy of low-oxidative-cost thermogenesis in prolonged cold adaptation.

4.2. Molecular evolution of the creatine cycle

The evolutionary path of CK-MT highlights the benefits of futile cycling. Mammalian CK-MT has evolved to include a C-terminal cardiolipin-binding motif, allowing it to securely anchor to mitochondrial inner membranes as supercomplexes. Cryo-EM analyses reveal pivotal amino acid substitutions at the CK-MT/ANT interaction interface in eutherians, enhancing complex stability and elevating PCr synthesis rates from 12 nmol $mg^{-1}\cdot min^{-1}$ in monotremes to 38 nmol $mg^{-1}\cdot min^{-1}$ in primates. This structural optimization allows creatine cycling to compensate for >50% of UCP1-mediated thermogenic capacity [25,63,64].

Genomic analyses further demonstrate strong positive selection for adipose creatine metabolism in mammals. Expansion of CREB-responsive elements in the *SLC6A8* promoter region (upstream -1.2 kb) enhances β3-adrenergic regulation of creatine uptake in eutherians [65,66]. Concurrently, brown adipose-specific enhancers evolved within *CK-MT* intronic regions, driving cold-induced *CK-MT* upregulation via PRDM16-mediated histone H3K27 acetylation [67]. Notably, CK-MT enhances mitochondrial respiration while suppressing ROS in white adipocytes and promoting cold-induced beige adipogenesis, a phenomenon observed in subcutaneous adipose depots [68]. These molecular innovations underscore CK-MT expression as a hallmark of cold adaptation under positive selection.

4.3. Creatine cycling and metabolic diseases

In white adipose tissue (WAT), obesity-induced endoplasmic reticulum (ER) stress activates the DNA methyltransferase DNMT3A via the IRE1-XBP1s pathway, leading to abnormal methylation of the CKB promoter and significantly inhibiting CKB transcription. This epigenetic silencing triggers creatine metabolism disorders. It promotes metabolic shift towards glycolysis, and on the other hand, it increases the secretion of pro-inflammatory chemokine CCL2, driving obesity-related inflammation [69]. Patients with type 2 diabetes (T2D) exhibit a metabolic imbalance characterized by elevated plasma creatine and reduced intramuscular phosphocreatine, the latter being directly associated with decreased expression of mitochondrial creatine kinase CKMT2. Silencing Ckmt2 significantly impairs mitochondrial respiration in muscle cells, while skeletal muscle-specific overexpression of CKMT2 can independently reverse mitochondrial dysfunction in high-fat diet mice regardless of creatine availability. Notably, exercise training effectively upregulates CKMT2 expression in human and mouse skeletal muscle, providing a key mechanistic basis for improving mitochondrial pathology in T2D [70].

There is significant controversy regarding the role of creatine cycling, particularly futile creatine cycling (FCC), in energy expenditure in adipose tissue. Although brain-type creatine kinase (CKB) is believed to stimulate thermogenesis in brown adipose tissue (BAT) through FCC, evidence for functional FCC in WAT is weak. Experiments show that creatine does not stimulate respiration in cultured fat cells, isolated mitochondria, or permeabilized WAT. Furthermore, knocking down mitochondrial creatine kinase (CKMT1) in fat cells or knocking out CKMT1 in mice did not affect basal or β3-adrenergic receptor agonist-stimulated energy expenditure, mitochondrial function, or susceptibility to metabolic damage induced by a high-fat diet [71]. These findings do not support the necessity of CKMT1 in the regulation of WAT energy expenditure and question the existence of FCC in WAT. However, at the level of treatment strategies, targeting creatine metabolism pathways shows promise. A novel salicylic acid nitroalkene derivative, SANA, can effectively improve obesity, hepatic steatosis, and insulin resistance in diet-induced obesity mouse models at an extremely low dose. Its core mechanism involves activating energy expenditure and thermogenesis dependent on PCr in adipose tissue, stimulating mitochondrial respiration. This effect depends on the presence of PCr and requires binding with CK isoenzymes CKMT1/2. Downregulation of CKMT1 interferes with the *in vivo* efficacy of SANA. Therefore, SANA, as a first-in-class drug, activates the phosphocreatine energy-consuming pathway and emerges as a potential candidate for combating 'sugar obesity' [72].

There remains debate around the role of creatine cycling in thermogenesis. Pharmacological inhibition of creatine kinase (such as using β-GPA) can induce compensatory thermogenesis and improve metabolism [73], and periodic creatine supplementation has been reported to enhance thermogenesis in human adipose tissue under mild cold exposure without significant side effects [74,75]. However, other studies have not observed any effect of creatine on cold-induced thermogenesis or BAT activity in young women, and the pronounced thermogenic effects in rodent models may involve muscle-specific mechanisms (such as β-receptor sensitivity, UCP-3 expression) and more complex factors in humans, such as absorption efficiency, individual differences in CK activity, and mitochondrial heterogeneity [76]. Overall, although there are positive findings such as CK-MT overexpression protecting mitochondrial function [77], there is currently insufficient convincing evidence to support the existence of a significant and universal futile creatine cycle in adipose tissue.

BAT is specifically responsible for non-shivering thermogenesis, achieving an unrestricted respiratory process by combining lipolysis, highly active mitochondrial electron transport chains, and a unique regulatory uncoupling protein UCP1. Similar to other tissues with high variable respiratory characteristics, BAT contains creatine pools, mitochondria, and cytosolic creatine kinase isoforms. The genetic and pharmacological regulation of these components appears to influence diet- and cold-induced metabolic responses in both *in vivo* and *in vitro* models. However, the clarity of its mechanisms and the effectiveness of therapeutic strategies remain unconvincing. [36]. Therefore, we urgently need a novel explanation for the high incidence of obesity-related metabolic diseases.

The rapid transition from cold adaptation to thermoneutral environments, driven by modern climate control, has precipitated UCP1 functional decay (contemporary human BAT activity is merely 17% of Neanderthal levels) while failing to engage compensatory creatine cycling [78]. Genome-wide association studies (GWAS) of creatine

metabolism-related genes reveal an obesity-linked SNP (rs1136165) in CKB, while the rare CK-MT1B allele rs149544188 exhibits anti-obesity effects. Rising obesity rates may reflect evolutionary mismatch: energy previously expended via UCP1-mediated thermogenesis in cold environments is preserved under thermal comfort [79]. In other words, humans' heat-generating abilities, which were acquired during evolution, cannot be utilized in air-conditioned environments, and the excess energy can only manifest as obesity. The evolutionary optimization of the FCC illuminates novel therapeutic paradigms. The shift from UCP1- to creatine-driven thermogenesis exemplifies the biological trade-offs between energy efficiency and oxidative damage. By minimizing ROS production through the organization of supramolecular complexes, creatine cycling confines thermogenic costs within safety thresholds, providing a biochemical blueprint not only for conquering cold but also for resolving the oxidative paradox in metabolic diseases.

5. THE SUPRAMOLECULAR MACHINERY OF VDAC/ANT-CK ASSEMBLY

The ultimate code for mitochondrial energy transfer resides in the supramolecular complex formed by VDAC (voltage-dependent anion channel), ANT (adenine nucleotide translocase), and CK-MT (Figure 2). Cryo-EM studies capture its voltage-gated assembly: at membrane potentials >150 mV, charge—complementary interfaces between CK-MT's N-terminal α-helix and VDAC's β-barrel domain create a 1.8 nm-diameter energy conduit. CK-MT interacts with phosphatidic acid, anionic phospholipids, and VDAC, stabilizing inner/outer membrane contact sites where ANT integrates into these proteolipid complexes. This molecular switch enhances PCr synthesis efficiency by 3.2-fold compared to free diffusion, revolutionizing our understanding of mitochondrial energy conduction [63].

New cryo-electron microscopy evidence indicates that the oligomeric state of the ATP synthase complex directly regulates the structure of mitochondrial cristae. This may, in turn, affect the substrate channeling efficiency of the CK system through curvature-dependent membrane protein sorting. Particularly under metabolic stress, the plasticity of cristae connections can dynamically regulate the coupling efficiency between VDAC-ANT-CK microdomains and ATP synthase arrays, which may explain the tissue-specific creatine-sensitive bioenergetic changes [80]. The topology of cristae acts as a spatial code for coordinating nucleotide transfer. In BAT mitochondria, closely packed cristae might enforce prioritized CK/ANT/ATP synthase crosstalk, while loosely arranged cristae in skeletal muscle can buffer this spatial coordination requirement. In skeletal muscle, the increase in proton potential generated by substrate oxidation enhances oxidative phosphorylation, and the flux control at ANT increases, making the dissociation of ADP-ANT binding easier [81]. This hypothesis aligns with recent studies by Brave et al. on membrane contact-driven metabolite channels [82], although further comparative analysis is needed to understand the tissue-specific ultrastructural differences of cristae.

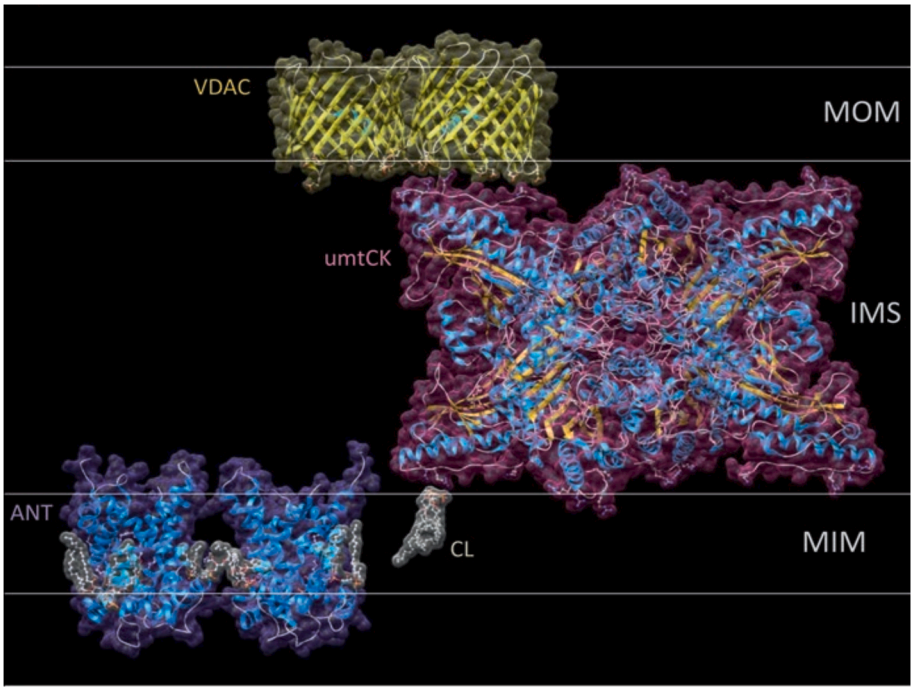

Figure 2: Putative topology of the VDAC/ANT-CK supramolecular machine. The cubic homooligomer umtCK (red surface) and its direct interaction partner VDAC (yellow surface) are embedded in phospholipid cardiolipin (CL, white surface) within the mitochondrial outer membrane (MOM) and inner membrane (MIM). ANT (magenta surface) is located near this complex in the MIM, and functional data and co-purification experiments indicate a close proximity between them, suggesting they reside within the same MIM phosphatidyl carnitine patch. Note that this figure only shows the interaction of one CL molecule and one VDAC dimer with one dimer of the umtCK octamer; however, each mtCK dimer may participate in such interactions, including additional interactions with other anionic phospholipids in the MOM. Additionally, VDAC and ANT likely form dimers (as shown) or higher-order oligomers *in vivo*, and this figure only depicts the crystal structure of the dimer (Schlattner et al. ,2018). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Time-resolved cryo-EM further reveals dynamic assembly mechanics. Membrane polarization >150 mV induces torsional deformation of CK-MT α-helix, triggering salt bridge formation with VDAC's β2-strand. The central channel, lined by CK-MT Lys28/Arg32 and VDAC's Asp54, forms a positively charged filter (−1 net charge selectivity), permitting directional PCr flux. Concurrently, ANT's matrix-side aperture narrows to exclude ATP^{4-} via steric hindrance while allowing ADP^{3-} entry, enforcing unidirectional adenylate cycling [83].

Molecular dynamics (MD) simulations elucidate PCr translocation mechanisms. Guanidino nitrogens in PCr molecule form hydrogen-bonded water chains, enabling proton shuttling via the Grotthuss mechanism (proton jumping mechanism). Face-to-edge π-stacking between PCr's creatine ring and CK-MT Trp79 elevates diffusion coefficients, while the local electric field accelerates PCr migration to 3. 8 × free diffusion rates [84]. This suggests that PCr may stimulate mitochondrial respiration. It is noteworthy that predictions by MD are contrary to the results observed by Walsh in isolated mitochondria. In isolated mitochondria, PCr reduces the sensitivity of mitochondrial respiration to ADP, while Cr has the opposite effect. During the transition from rest to high-intensity exercise, the decrease in the PCr/Cr ratio effectively increases the sensitivity of mitochondrial respiration to ADP [20]. Ydfors et al. also emphasized the importance of simulating the *in vivo* Cr/PCr ratio, as the presence of Cr directly influences or even reverses mitochondrial respiratory sensitivity to ADP [85]. Another study on permeabilized muscle fibers further refined the factors affecting the kinetics of ADP-stimulated mitochondrial respiration, including contractile state (including myosin-ATPase), temperature, and oxygen partial pressure. These conditions significantly alter the Km for ADP and mitochondrial respiratory efficiency [86]. Notably, the contractile state was identified as a critical factor that dynamically optimizes mitochondrial respiration to match muscular energy demands. Consequently, the MD designed to predict mitochondrial respiration and the potential role of PCr constructed merely an idealized, singular scenario (excluding Cr and myosin-ATPase), which significantly diverged from actual physiological conditions and yielded inconsistent results. In addition, supramolecular activity is dynamically regulated by ATP/PCr ratios. PCr >25 mM stabilizes open conformations via Arg223 salt bridges, whereas ADP^{3-} binding to ANT's Arg159 reduces complex half-life [87]. Oxidative modification (ANT Cys sulfonation by H_2O_2) enlarges channel diameter, permitting ATP^{4-} leakage and impairing oxidative phosphorylation [88].

In type II muscle fibers, VDAC/ANT-CK complexes reach densities of 1,200 per μm^2, achieving millisecond-level ATP transfer efficiency through channel-confined diffusion, which meets the immediate energy needs of sarcoplasmic reticulum Ca^{2+}-ATPase. This 'energy conduit' formed by such complexes is particularly significant in fast-twitch muscle fibers, closely related to the rapid contraction-recharging process during high-intensity exercise [89]. PCr in the cytoplasm can directly convert ADP into ATP within the mitochondrial matrix through the VDAC/ANT-CK complexes. Subsequently, the ATP released by the ADP exchange via the unbound ANT portion is utilized by the outer membrane VDAC-hexokinase electrogenic complex to convert cytoplasmic glucose into glucose-6-phosphate within the intermembrane space of the mitochondria [90]. Pathologically, membrane depolarization (<110 mV) in heart failure disassembles these complexes, delaying ATP supply and impairing contraction [91]. Oxidative crosslinking of ANT's Cys159/Cys256 further inhibits complex reassembly [92]. These findings reveal nature's nanoscale engineering principles for precise energy transfer, a paradigm with transformative implications for the treatment of bioenergetic disorders.

6. PHASE SEPARATION OF CK CONDENSATES AND SPATIOTEMPORAL METABOLIC REGULATION

Recent paradigm-shifting studies reveal a novel regulatory dimension of CK activity through liquid—liquid phase separation (LLPS, Figure 3). This spatiotemporal mechanism enables CK to form dynamic condensates that transiently couple kinase—substrate interactions, creating localized "energy reactors" for precision metabolic control [93]. Molecular dynamics simulations indicate that CK undergoes LLPS at PCr >25 mM to form membrane-associated condensates at microdomains of the mitochondrial inner membrane, which are enriched with ANT and CK-MT. These proteolipid platforms, composed of phosphatidylcholine, phosphatidylethanolamine, and cardiolipin in a 2:1:1 M ratio, enhance localized ATP regeneration rates through substrate channeling [94,95]. Annexin A2-mediated anchoring of CK condensates near myofibrillar Z-lines further optimizes ATP delivery to myosin heads, achieving millisecond-scale energy matching during contraction [96].

It is particularly noteworthy that ATP, beyond its conventional role as a currency of cellular energy, serves as a hydrotropic agent that regulates LLPS. At mM concentrations, ATP impedes protein aggregation and simultaneously promotes phase separation by engaging in π-cation interactions and modulating hydration levels. This multifaceted capability fortifies condensates of CK by stabilizing them without the necessity of direct binding, functioning as a molecular "wedge" that coordinates the dynamics of macromolecular crowding [97]. Experimental models using semi-permeable polymeric vesicles confirm that spatial confinement of CK and ADP within phase-separated compartments accelerates enzymatic cascades, challenging classical homogenous solution kinetics [98].

CK condensate dysregulation underlies exercise adaptation and multiple pathologies. During high-intensity interval training (HIIT), type II muscle fibers exhibit transient PCr spikes (32 mM) that trigger CK phase separation, enabling 9-fold faster ATP regeneration to sustain peak power output [99]. Chronic training expands condensate volume, delaying PCr depletion despite minimal absolute increases in PCr. Post-eccentric contraction, HSP70-enriched condensates accelerate CK refolding, facilitating muscle repair [100]. In heart failure, myocardial PCr depletion reduces condensate integrity, impairing ATP supply and contractility. Elevated PCr/ATP ratios in insulin-resistant adipocytes induce pathological phase separation, exacerbating lipotoxicity in metabolic disorders. Reduced cerebral PCr disrupts synaptic ATP provisioning, promoting tau phosphorylation and cognitive decline [101]. These findings position the CK phase dynamics as a master regulator of bioenergetic plasticity.

Muscle contraction requires high energy flux, which is provided by muscle-type CK (MM-CK) that couples with myofibrils. MM-CK can physically interact with slow skeletal muscle myosin binding protein C1 (MyBPC1) in a dose-dependent manner dependent on creatine concentration but independent of ATP, ADP, or PCr. MyBPC1 acts as an adapter connecting ATP consumers (myosin) and regenerators (MM-CK) to achieve efficient energy metabolism and homeostasis [102]. Another study shows that in the hearts of cold-blooded vertebrates, CK bound to myofibrils is necessary for fully activating myosin-ATPase activity, although their energy conversion rate is lower compared to homeothermic animals [103]. Further studies have shown that CK-MT-induced CL separation occurs on a bilayer model forming micrometer-sized lipid domains, which only appear when the

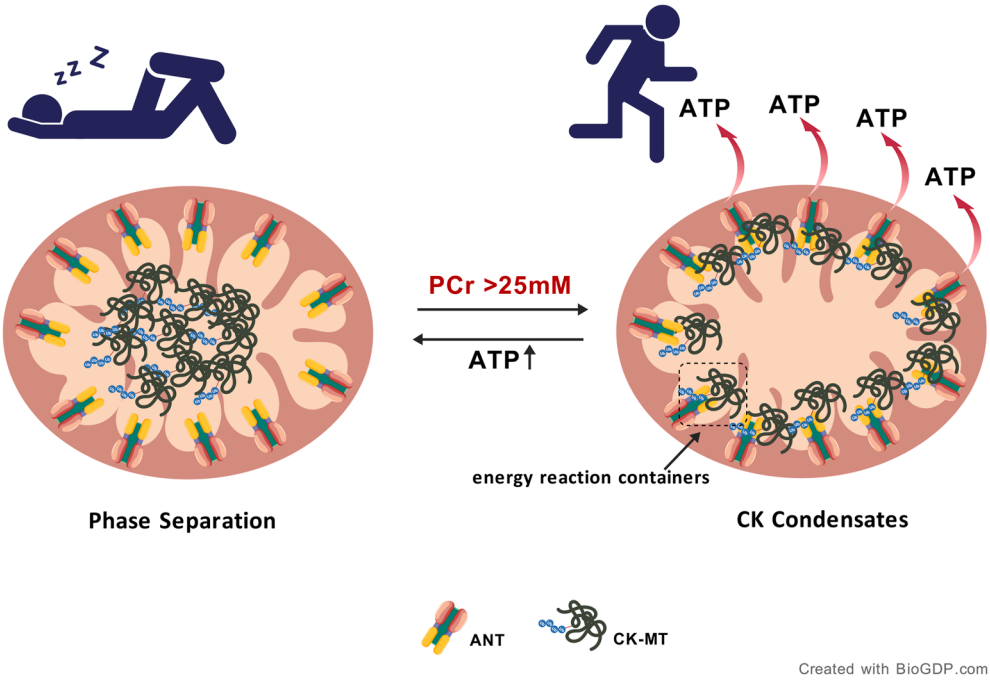

Figure 3: Phase separation of CK condensates. This spatiotemporal mechanism allows creatine kinase CK to form dynamic condensates, temporarily coupling CK kinase with its substrate ADP, creating localized 'energy reaction containers'. Molecular dynamics simulations show that when PCr concentration exceeds 25 mM, CK undergoes liquid—liquid phase separation and assembles into membrane-associated condensates within mitochondrial inner membrane microdomains rich in ANT and CK-MT. These protein-lipid platforms composed of phosphatidylcholine, phosphatidylethanolamine, and cardiolipin rapidly increase local ATP regeneration through substrate channels. Given the significant reliance of this process on physical phase transitions, it is markedly faster than complex biochemical reactions, making it more suitable for the sudden energy demands encountered during exercise.

lipid mixture contains CL, accompanied by protein multimer assembly, vesicle entrapment, as well as changes in vesicle curvature and membrane fluidity. These results highlight the importance of highly enriched CK-MT in the lateral organization of the inner mitochondrial membrane [104]. Reassembling mammalian mitochondrial complex I into phospholipid nanodiscs containing exogenous Q10, then using Cryo-EM reveals that in the 'active' resting state of the complex, one Q10 molecule occupies the entire Q-binding site, along with a matching substrate-free structure [105]. These studies further delineate the relationship between PCr/Cr shuttling, ultrastructural organization of muscle fibers, and myosin, providing a deeper depiction of energy production and distribution pathways. We even predict that these molecular behaviors may also be a part of LLPS, although there is currently no conclusive observational evidence.

7. THE CREATINE CYCLE IN THERAPEUTIC STRATEGIES FOR METABOLIC DISORDERS

The creatine cycle is an energy buffering system shared by all cells and tissues, exhibiting high conservation across species. The creatine kinase (CK) system converts ATP into PCr for storage, offering new therapeutic approaches for metabolic diseases [106]. Targeting the creatine cycle, particularly the phase transition process of CK condensates, is expected to redistribute or consume excess energy within the body, thereby reducing fat accumulation.

To achieve this goal, we must confront and surmount numerous challenges. While existing studies indicates that the targeted activation of the creatine cycle could influence energy metabolism and diminish fat accumulation [32,107], the precise molecular mechanisms and signaling pathways are not yet fully understood. For instance, how the creatine cycle modulates the metabolic functions of adipose cells, and the potential ripple effects on other tissues and organs, requires further studies. Moreover, genetic background, metabolic rate, and lifestyle factors can introduce significant variability in the outcomes of targeted creatine cycle activation across different populations [108]. Technically, there is a current deficit in efficient and specific targeting methods, complicating the precise targeting of the creatine cycle and potentially leading to suboptimal activation or unintended off-target effects, which could affect the overall therapeutic efficacy.

Targeting condensate homeostasis through pharmacological chaperones or phase-modulating compounds holds promise for treating diabetes, obesity, and neuromuscular disorders [109]. The condensates formed by liquid—liquid phase separation (LLPS) of CK protein in specific microenvironments can significantly enhance energy metabolic flux by 3—5 times [93]. This self-organization phenomenon of biomacromolecules opens up new approaches for targeted regulation of energy metabolism. The core of phase transition regulation of CK condensates lies in the dynamic interaction between its N-terminal domain and ATP/ADP concentrations. When intracellular ATP concentration exceeds the threshold, CK monomers aggregate into catalytically active condensates through charge interactions, which can instantaneously increase the efficiency of PCr synthesis [110]. The rs72613567 polymorphism in 17-beta hydroxysteroid

dehydrogenase 13 (HSD17B13) has been found to reduce the progression from steatosis to metabolic dysfunction-associated steatohepatitis (MASH). HSD17B13 forms liquid—liquid phase separations (LLPS) around lipid droplets in the livers of MASH patients. Recent studies indicate that the LLPS of HSD17B13 triggers liver inflammation by promoting leukocyte adhesion [111]. Another study found that long-chain non-coding RNA-MEG3 (lncRNA-MEG3) regulates muscle mass and metabolic homeostasis by promoting SUZ12 liquid—liquid phase separation. lncRNA-MEG3 is preferentially expressed in slow-twitch muscle fibers and is crucial for maintaining muscle mass and function. This study revealed a new interaction between lncRNA-MEG3 and the polycomb repressive complex 2 (PRC2), where lncRNA-MEG3 binds to the PRC2 subunit SUZ12, promoting SUZ12 liquid—liquid phase separation, thereby maintaining metabolic homeostasis [112]. Animal studies targeting CK phase transition have not been reported yet, but the two cases mentioned above provide a window for treating metabolic diseases by targeting CK phase transition.

Targeted metabolic disease treatment strategies aimed at CK phase transitions still face several technical bottlenecks. First is the issue of dynamic equilibrium control during the phase transition process. The formation of kinase condensates is regulated by the AMPK/mTORC1 signaling axis, but existing small-molecule modulators have a half-life of about 2—3 h, which fails to meet the kinetic requirements of phase transitions [113,114]. Second is the problem of tissue targeting; studies showed significant differences in CK subtype expression between liver and fat tissues, raising higher demands for precise drug targeting in design [115]. Finally, there are challenges in developing delivery systems; traditional lipid-based carriers have insufficient enrichment efficiency in muscle tissues, while new cationic nanoparticles, although they can improve delivery efficiency, pose risks of liver and kidney toxicity. These challenges necessitate innovative approaches to enhance the delivery of therapeutic agents to target tissues while minimizing off-target effects and potential toxicities [116].

8. CONCLUSIONS AND PERSPECTIVES

Creatine has expanded from its traditional role as an exercise enhancer to a key regulator of systemic metabolism. Beyond maintaining ATP balance through the phosphocreatine system for high-intensity energy, new evidence shows creatine as a major coordinator of energy exchange between organs. In skeletal muscle, creatine links mitochondrial oxidative phosphorylation and glycolysis, facilitating targeted energy transfer via mitochondrial supercomplexes (VDAC/ANT-CK). In adipose tissue, creatine metabolism boosts the thermogenic capacity of brown adipocytes, enhancing energy dissipation and metabolic flexibility. The identification of the BLSA metabolic axis highlights creatine broader therapeutic potential, suggesting new approaches to address obesity, type 2 diabetes, mood disorders and age-related neurodegenerative diseases.

The future studies can focus on: (1) interpreting the regulatory impact of creatine on basal metabolism within non-exercise metabolic networks, particularly its molecular interactions with energy sensors such as the AMPK/mTOR axis in driving metabolic reprogramming. (2) Elucidating the mechanism of creatine-mediated interorgan energy communication: how skeletal muscle and adipose tissue exchange metabolic signals through creatine flux. (3) Revealing the biophysical principles underlying CK condensate formation and their adaptive roles in exercise metabolism, potentially guiding the development of phase-targeted therapies. This paradigm shift requires interdisciplinary collaboration to translate mechanistic insights into clinical innovations. By addressing these knowledge gaps, we may unlock the full potential of creatine as a metabolic hub connecting cellular bioenergetics, systemic homeostasis, and therapeutic discovery.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Yuhui Su: Writing — review & editing, Writing — original draft.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY

No data was used for the research described in the article.

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