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# Lac-Phe: An Exercise Hormone for Metabolic Regulation

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## Keywords

exercise, *N*-lactoyl-phenylalanine, CNDP2, energy balance, hypothalamic neural circuits, clinical applications

## Abstract

*N*-Lactoyl-phenylalanine (Lac-Phe), a conserved exercise-induced metabolite that rises rapidly in circulation, has emerged as a key molecular mediator linking fundamental metabolism to translational medicine. In this review, we elaborate recent findings that define a two-step framework for Lac-Phe biosynthesis and excretion. Functional studies show that Lac-Phe exerts broad metabolic benefits and should be regarded as a bioactive molecule rather than a passive by-product of intermediary metabolism. Furthermore, we review the neural mechanisms through which Lac-Phe conveys peripheral metabolic cues to central circuits regulating appetite and energy balance. Beyond the role of Lac-Phe in energy balance, alterations in Lac-Phe concentrations are associated with disease pathogenesis and progression, underscoring its potential as both a biomarker and a therapeutic agent. Collectively, these advances position Lac-Phe at the intersection of exercise physiology, metabolism, and disease and thus highlight its potential to integrate metabolic and physiological health.

## Contents



## 1. INTRODUCTION

Exercise is widely recognized as a foundation of preventive health and is consistently associated with reduced risk of cardiovascular disease, type 2 diabetes mellitus, and all-cause mortality (77, 108). Beyond these clinical outcomes, physical activity improves vascular function, insulin sensitivity, and lipid metabolism, underscoring the value of exercise as a nonpharmacological strategy for promoting metabolic health and longevity (27, 72). However, the molecular mechanisms linking exercise to its physiological benefits remain incompletely understood (87). This knowledge gap has driven efforts to identify circulating metabolites, hormones, and neural circuits that mediate the effects of exercise, with recent advances in metabolomics proving especially transformative. Unbiased profiling has highlighted not only classical exercise-induced metabolites such as lactate and succinate but also a range of previously unrecognized molecules (67). Among these, *N*-lactoyl-phenylalanine (Lac-Phe) has emerged as one of the most robustly induced factors across species, including mice, humans, and even racehorses subjected to exercise (58). Its rapid, transient elevation following exercise suggests that Lac-Phe functions as a signaling cue linking muscular activity to systemic metabolic regulation.

Here, we review the Lac-Phe biosynthesis and its transporters, which together define a two-step framework for Lac-Phe production and excretion. Accumulating evidence shows that Lac-Phe exerts diverse metabolic benefits and should be viewed as far more than a passive by-product of intermediary metabolism (**Figure 1**). Acting as a peripheral cue, Lac-Phe communicates metabolic state to central pathways that coordinate appetite and energy balance (**Figure 2**). Beyond the metabolic roles of Lac-Phe, alterations in Lac-Phe levels have been associated with a range of pathological conditions, highlighting its potential as a biomarker for early disease detection (**Figure 3**). Finally, we discuss the emerging physiological and therapeutic functions of Lac-Phe, along with future directions for elucidating its mechanisms and advancing its translational applications.

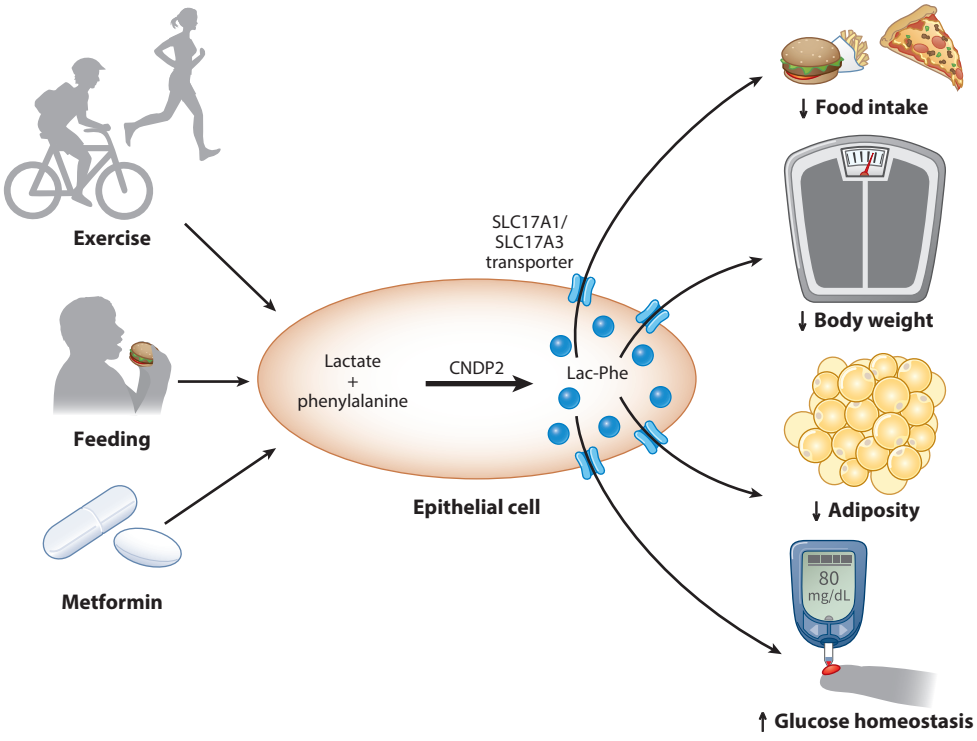


**Figure 1**

Pathways of Lac-Phe biosynthesis and excretion and its metabolic benefits. Exercise, feeding, and metformin elevate circulating Lac-Phe. Specifically, in epithelial cells, Lac-Phe is synthesized by CNDP2 from lactate and phenylalanine and then exported via the transporters SLC17A1 and SLC17A3. In turn, increased serum Lac-Phe elicits sustained reductions in food intake, body weight, and adiposity while concurrently improving glucose homeostasis. Abbreviations: CNDP2, cytosolic nonspecific dipeptidase 2; Lac-Phe, *N*-lactoyl-phenylalanine.

## 2. Lac-Phe PRODUCTION PATHWAY

### 2.1. Lac-Phe as an Exercise-Induced Metabolite

Regular physical activity confers a broad spectrum of health benefits, including reduced risk of cardiovascular disease, type 2 diabetes mellitus, and obesity (77, 108, 109). By contrast, physical inactivity markedly increases susceptibility to metabolic disorders and elevates all-cause mortality (35, 81). These well-established associations have motivated considerable effort to uncover the molecular mechanisms that translate exercise into systemic metabolic improvements. In particular, the research has focused on identifying circulating metabolites that act as signaling intermediates between contracting muscle and peripheral organs (87).

Using both targeted and untargeted metabolomic profiling to analyze plasma from mice subjected to an acute bout of exhaustive treadmill running, Li and colleagues (58) identified Lac-Phe, a conjugate of lactate and phenylalanine, as the most strongly induced metabolite. Notably, Lac-Phe concentrations in mouse plasma increased sharply immediately after exercise and declined to baseline within approximately 1 h (58). However, intracerebral measurements in mice reveal that Lac-Phe within the arcuate nucleus of the hypothalamus (ARH) persists for up to 2 h following intraperitoneal (i.p.) administration, suggesting a longer residence time in the brain (61).

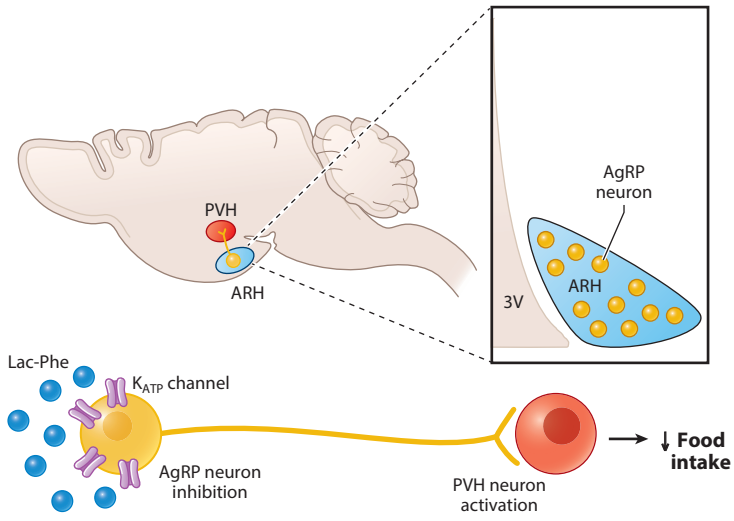


**Figure 2**

Neural mechanisms underlying Lac-Phe-induced hypophagia. Lac-Phe directly inhibits AgRP neurons in the ARH via activation of $K_{ATP}$ channels. This inhibition of AgRP neurons, in turn, activates neurons in the PVH, thereby shifting hypothalamic network activity toward satiety and ultimately reducing food intake. Abbreviations: 3V, third ventricle; AgRP, Agouti-related peptide; ARH, arcuate nucleus of the hypothalamus; $K_{ATP}$ channel, ATP-sensitive potassium channel; Lac-Phe, *N*-lactoyl-phenylalanine; PVH, paraventricular nucleus of the hypothalamus.

These findings imply that Lac-Phe bioavailability varies across tissues in both concentration and duration, highlighting the need for systematic investigation of its organ-specific kinetics. These dynamics indicate that Lac-Phe functions as a rapid and transient molecular signal that is capable of linking acute muscular activity with downstream metabolic adaptations.

Pharmacological studies have reinforced this hypothesis by demonstrating functional relevance. The i.p. injection (50 mg $kg^{-1}$) of Lac-Phe to mimic postexercise concentrations in diet-induced obese (DIO) mice potently suppressed feeding behavior (58). With repeated administration, Lac-Phe produced chronic hypophagia accompanied by reductions in body weight and adiposity as well as improved glucose tolerance (6, 58, 66). Importantly, these effects occurred without changes in energy expenditure or locomotor activity, suggesting that Lac-Phe primarily acts through regulating food intake rather than altering energy utilization. Together, these findings identify Lac-Phe as a candidate mediator of the appetite-suppressive and metabolic benefits of exercise.

Large, activity-inducible surges in Lac-Phe have been observed not only in rodents but also in humans and racehorses, highlighting an evolutionarily conserved role for this metabolite across mammals (55, 58, 63). In human studies, Lac-Phe responses appear to scale with exercise intensity: Sprinting induces the largest increases, resistance exercise produces intermediate responses, and endurance training results in more modest elevations (58). This intensity dependence suggests that Lac-Phe may serve as a sensitive biomarker of vigorous muscular activity. Moreover, it implies that moderate-intensity exercise, although beneficial for other aspects of health, may not provide sufficient stimulus for substantial Lac-Phe accumulation (109).

The rapid, transient induction of Lac-Phe after exercise; its robust anorexigenic effects in obese animals; and its conservation across species underscore its potential role as a molecular effector of exercise benefits (**Figure 1**). At the same time, the intensity-dependent response pattern suggests that Lac-Phe could serve as a useful biomarker for evaluating the quality and effectiveness of

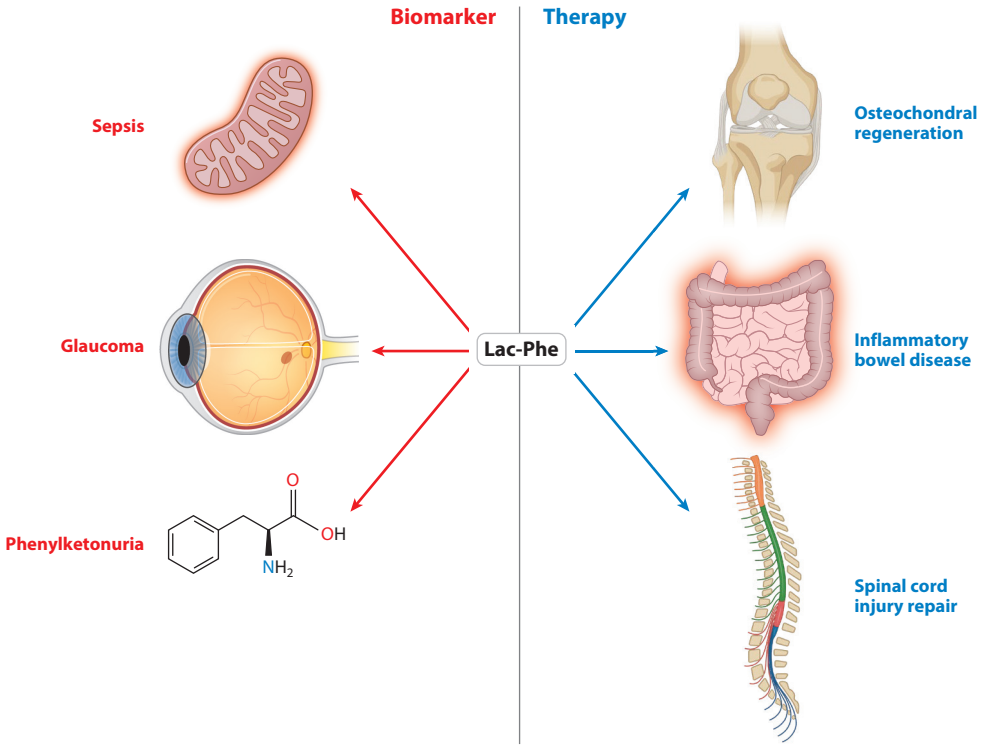


**Figure 3**

Lac-Phe (*N*-lactoyl-phenylalanine) as a biomarker and therapeutic candidate. Emerging evidence indicates that Lac-Phe may serve as a clinically relevant biomarker in sepsis, glaucoma, and phenylketonuria, where altered circulating levels track disease risk or progression. In parallel, preclinical studies suggest therapeutic potential: Lac-Phe improves osteochondral regeneration, attenuates inflammatory bowel disease, and enhances recovery after spinal cord injury. Figure adapted from images created in BioRender; Liu Q. 2026. **https://BioRender.com/7n36h49**.

physical activity. Further research will be needed to define the precise mechanisms by which Lac-Phe exerts its effects and to determine how its biology can be leveraged for therapeutic strategies aimed at combating obesity and metabolic disease.

## 2.2. Regulation of Lac-Phe by Feeding Behavior

In addition to the modulation of Lac-Phe concentrations by exercise, accumulating evidence demonstrates that feeding behavior is a key determinant of circulating Lac-Phe concentrations (69). In mice, plasma Lac-Phe levels are markedly suppressed during fasting but rise rapidly following refeeding (113). These dynamics indicate that Lac-Phe is acutely sensitive to nutrient availability (**Figure 1**). Consistent with this interpretation, human studies have shown that serum Lac-Phe concentrations are significantly higher in the fed state compared with fasting, reinforcing the view that food intake is a primary driver of Lac-Phe fluctuations (90).

Beyond the binary distinction between fasting and feeding, Lac-Phe regulation also depends on diet form. For instance, volunteers who consumed sugar-rich fruits exhibited a more pronounced postprandial rise in Lac-Phe compared with those ingesting liquid sugar (90). This divergence implies that solid versus liquid meals engage partially distinct metabolic or absorptive pathways that differentially influence Lac-Phe production. From a physiological standpoint, these results raise

the possibility that Lac-Phe contributes to satiety differences observed between food matrices, thereby linking nutrient form to appetite regulation. Such observations are particularly relevant given the increasing prevalence of liquid calories in modern diets, which are often associated with impaired satiety responses.

Feeding behavior also interacts with circadian rhythms to shape Lac-Phe availability. In rats, high-calorie-diet consumption under time-restricted feeding paradigms induces time-of-day-dependent alterations in brain Lac-Phe levels even in the absence of exercise (41). In these models, hypothalamic Lac-Phe concentrations in rats fluctuate across the light–dark cycle and tend to be higher during the scheduled feeding phase than during the fasting phase (41). However, it remains unclear whether these day–night differences in Lac-Phe reflect an intrinsic circadian rhythm, periprandial changes in nutrient availability, or a combination of both. Thus, current data support the idea that Lac-Phe is positioned at the interface of feeding schedules and circadian timing, but more work is needed to distinguish between feeding-dependent and clock-driven components of this rhythm.

Overall, these findings broaden our understanding of Lac-Phe as more than a simple exercise-induced metabolite. Its appetite-suppressing properties, combined with robust postprandial elevation, support the hypothesis that Lac-Phe acts as a feedback signal to restrain food intake following meals (90). Thus, its sensitivity to dietary form and circadian timing suggests that Lac-Phe integrates multiple layers of nutritional and temporal information to fine-tune energy balance.

## 2.3. Lac-Phe Biosynthesis from Lactate by CNDP2

The biochemical synthesis of Lac-Phe has become increasingly well-characterized in recent years, with lactate and phenylalanine identified as its two essential precursors (40, 58, 73). Traditionally, lactate was regarded as a glycolytic by-product that accumulates under conditions of high energy demand, such as during muscle contraction in exercise, when circulating concentrations can rise to millimolar levels (9, 28). Lactate is currently recognized as a multifunctional metabolite with systemic influence: It fuels oxidative metabolism, maintains tissue redox balance through intercellular shuttling, functions as a signaling ligand via cell surface receptors, and contributes to posttranslational protein modifications (38, 60, 119, 120). Therefore, lactate emerges not simply as a waste product but as a central hub of metabolic communication, providing the biochemical foundation for Lac-Phe synthesis.

Phenylalanine, the other precursor, is an essential amino acid that plays a classical role in protein synthesis. In human studies, pre-exercise supplementation with phenylalanine enhances whole-body fat oxidation during exercise (103), underscoring the contribution of phenylalanine to energy regulation. The formation of Lac-Phe from lactate and phenylalanine therefore represents a nontraditional biochemical intersection between glycolytic metabolism and amino acid metabolism, bringing together two pathways that typically operate independently and generating a metabolite with signaling properties distinct from those of either precursor. Using whole-cell lysate in HEK293 cells, Jansen et al. (40) demonstrated that coincubating lactate and phenylalanine with nondenatured proteins generated Lac-Phe, implicating an enzymatic process. Subsequent investigations identified cytosolic nonspecific dipeptidase 2 (CNDP2) as the enzyme catalyzing the condensation of lactate and phenylalanine into Lac-Phe (40, 58, 112).

CNDP2, also known as CPGL (carboxypeptidase of glutamate-like) or carnosine dipeptidase II, belongs to the M20 family of metallopeptidases (98). Beyond the canonical enzymatic functions of CNDP2, emerging evidence points to its broader physiological roles. For example, its involvement in regulating oxidative stress has been demonstrated in both mice (48) and fruit flies (115), suggesting a conserved function across species. CNDP2 has also been implicated in

metabolic disease, as it may contribute to the development of posttransplantation diabetes mellitus, a common complication following kidney transplantation (44). In addition, studies in cancer models reveal that CNDP2 can activate the PI3K/AKT and MAPK signaling pathways, can upregulate key cell cycle regulators such as cyclin E and cyclin B1, and can interact with pepsinogen C (73). Together, these findings underscore the diverse biological contexts in which CNDP2 operates, ranging from oxidative stress responses to metabolic dysfunction and tumorigenesis. Several common human polymorphisms have been reported within the CNDP2 locus. Among these, the rs7577 single-nucleotide polymorphism (SNP) has been associated with an increased risk of diabetic nephropathy, particularly in women, and has been linked to altered kidney function. Individuals carrying the CC genotype exhibit lower CNDP2 expression and a correspondingly higher susceptibility to this condition (2). Beyond disease risk, CNDP2 variation has also been examined in the context of physical performance. Notably, the rs6566810 SNP, specifically the A/A genotype, is overrepresented in endurance athletes, although this enrichment appears limited to individuals competing at the international level (33). Together, these observations point to a potentially broad physiological relevance of CNDP2 genetic variation; however, the functional consequences of these polymorphisms remain largely unresolved.

In further support of the broad physiological relevance of CNDP2, it is highly conserved across species and shows strong expression in myeloid cells as well as epithelial tissues of the gut and kidney (98, 112). In CNDP2-deficient macrophage cell lines generated by CRISPR–Cas9, extracellular Lac-Phe levels were markedly reduced, demonstrating that CNDP2 is required for Lac-Phe biosynthesis. Supplementation with lactate increased Lac-Phe production, highlighting the importance of precursor availability (58). Additional insights come from the central nervous system (CNS). CNDP2 is abundantly expressed in microglia, suggesting that the brain may represent an additional site of Lac-Phe synthesis and regulation (41, 123). This observation broadens the potential functional significance of Lac-Phe beyond peripheral metabolism, hinting at a role in neuroimmune processes.

Circulating Lac-Phe concentrations are significantly lower in CNDP2-knockout (KO) mice compared with wild-type controls, both at baseline and after acute treadmill running (58). When placed on a high-fat diet, wild-type and CNDP2-KO mice exhibit similar food intake and body weight, indicating that reductions in basal Lac-Phe are not sufficient to alter energy balance. This observation prompted investigators to consider that an additional metabolic stimulus might be needed to reveal a phenotype associated with CNDP2 deficiency. Indeed, when the mice were subjected to chronic treadmill training in combination with high-fat-diet feeding, CNDP2-KO mice displayed increased food intake, accelerated weight gain, and greater adiposity relative to controls. These findings suggest that genetic ablation of Lac-Phe biosynthesis leads to increased energy intake and weight gain in the context of exercise training, but not in the sedentary state (58, 112). Collectively, these results establish CNDP2 as the principal biosynthetic enzyme responsible for both basal and exercise-inducible Lac-Phe production, underscoring its critical role in mediating the antiobesity effects of exercise.

The central role of CNDP2 has prompted interest in strategies to enhance its activity for therapeutic benefit. Berkel & Cacan (6) reported that treatment with *trans*-10, *cis*-12 conjugated linoleic acid—a naturally occurring dietary *trans* fatty acid—as well as dietary calorie restriction increased CNDP2 expression in adipose tissue in mice and rats. These interventions were associated with reduced food intake, body weight, and fat mass, effects that may be mediated, at least in part, by enhanced Lac-Phe biosynthesis (6, 69). Notably, CNDP2 expression declines with age in rats (6). Although age-related reductions in Lac-Phe have not been directly demonstrated, diminished CNDP2 expression suggests that Lac-Phe biosynthesis may also decrease with aging. Therefore, such changes may contribute to age-associated metabolic decline.

Collectively, these findings highlight CNDP2 as the foundation of Lac-Phe biosynthesis while simultaneously revealing important gaps in knowledge. The dual requirement of lactate and phenylalanine situates Lac-Phe at the nexus of energy metabolism and amino acid biology, while the role of CNDP2 links its synthesis to immune, epithelial, and potentially neural systems (**Figure 1**). Ultimately, a deeper mechanistic understanding of Lac-Phe biosynthesis will be critical for evaluating its translational potential as a therapeutic target for obesity and metabolic disease.

## 2.4. SLC17A1/3 Transporters Mediate Excretion of Lac-Phe

In recent years, the mechanisms underlying Lac-Phe transport have become increasingly well-characterized (41, 69). Understanding how Lac-Phe enters the circulation is critical, as its hypophagia effects depend on reaching target tissues beyond its site of origin. Accumulating evidence now supports the presence of dedicated transporters that actively mediate Lac-Phe efflux from cells, rather than passive diffusion. First, CNDP2, the enzyme responsible for Lac-Phe biosynthesis, functions in the cytosol, whereas Lac-Phe is consistently enriched in the conditioned medium of RAW264.7 macrophage cells (58). This finding implies that active export mechanisms must move Lac-Phe away from its intracellular site of production. Second, Lac-Phe is a polar metabolite, making passive diffusion across lipid bilayers energetically unfavorable and thereby strengthening the case for transporter-mediated efflux. Third, Lac-Phe can indeed be actively translocated across the plasma membrane. For example, overexpression of ABCC5 in HEK293 cells, a member of the ABC (ATP-binding cassette) transporter family, markedly increased Lac-Phe accumulation in the extracellular medium (40). However, despite this effect in vitro, Lac-Phe concentrations were not altered in ABCC5-deficient mice (58), indicating that ABCC5 is unlikely to represent the physiologically relevant transporter in vivo and that additional carriers remain to be identified.

Among candidate transporter families, members of the solute carrier (SLC) group, specifically SLC17A1–4, have received particular attention. These proteins were initially characterized as sodium–phosphate cotransporters. However, subsequent research revealed that they can also transport other small molecules such as urate and the organic anion *p*-aminohippuric acid (39, 75, 100). This broader substrate specificity, combined with their renal and hepatic expression patterns, made SLC17A1–4 strong candidates for Lac-Phe transport. Consistent with this, large-scale human genetic association studies linking metabolomic variation to genetic loci have implicated SLC17A1–4 in the regulation of dozens of endogenous and xenobiotic metabolites (89).

Direct experimental evidence further implicates SLC17A1 and SLC17A3 as physiologically relevant Lac-Phe transporters. Li and colleagues (59) reported that Lac-Phe was the most strongly elevated metabolite in the conditioned medium of SLC17A1-transfected HEK293T cells and the second-most-elevated metabolite in SLC17A3-transfected HEK293T cells. Interestingly, overexpression of CNDP2 alone in HEK293T cells failed to increase extracellular Lac-Phe, indicating that enhanced intracellular biosynthesis is not sufficient to drive Lac-Phe secretion (59). By contrast, overexpression of SLC17A3 and, to a lesser extent, SLC17A1 was sufficient to promote Lac-Phe efflux. Most strikingly, coexpression of CNDP2 with either SLC17A1 or SLC17A3 yielded dramatic synergistic effects, producing approximately 3-fold and 40-fold increases in extracellular Lac-Phe, respectively (59). These findings underscore a critical principle: Robust extracellular accumulation of Lac-Phe requires both efficient intracellular biosynthesis and transporter-mediated efflux, highlighting a cooperative interplay between CNDP2 and SLC17 family members.

In vivo studies further corroborate these findings and establish their physiological relevance. Urinary Lac-Phe levels show strong genetic associations with the SLC17A1–4 locus in humans

(59). In addition, targeted deletion of either SLC17A1 or SLC17A3 in mice led to significant reductions in urinary Lac-Phe concentrations, confirming that these transporters function in renal excretion of Lac-Phe (59). Overall, these findings firmly establish SLC17A1 and SLC17A3 as physiological transporters responsible for Lac-Phe excretion in vivo (**Figure 1**).

Although existing evidence strongly supports the role of SLC17A1 and SLC17A3 in Lac-Phe transport, additional mechanistic studies will be required to provide more definitive proof. In particular, reconstituted transport assays using purified SLC17 proteins will be needed both to determine whether these transporters directly and specifically carry Lac-Phe across membranes and to quantify their transport kinetics. Moreover, it remains to be clarified whether SLC17 transporters act exclusively in renal excretion or whether they also contribute to Lac-Phe distribution to other tissues, such as the brain and gut.

In summary, recent progress has delineated a two-step model of Lac-Phe handling: CNDP2 catalyzes intracellular biosynthesis, and SLC17 family transporters mediate Lac-Phe efflux and excretion. This integrated view emphasizes that Lac-Phe homeostasis depends on the coordination of production and transportation, and it highlights new avenues for research aimed at understanding how Lac-Phe levels are regulated across physiological and pathological states.

## 3. Lac-Phe IN REGULATION OF ENERGY METABOLISM

### 3.1. Lac-Phe Suppresses Feeding and Obesity

The discovery that Lac-Phe is the most prominently elevated metabolite in plasma following acute, high-intensity exercise in mice has prompted intensive investigation into its potential role as a signaling mediator in energy balance regulation. Initial pharmacological studies provided compelling evidence. When administered acutely via i.p. injection (50 mg $kg^{-1}$), Lac-Phe significantly suppressed food intake in DIO mice over a 12-h period (58). Notably, these reductions in feeding occurred without changes in energy expenditure or locomotor activity, indicating that Lac-Phe's anorexigenic actions are not secondary to generalized metabolic suppression or reduced physical activity. Rather, they appear to have a specific satiety-promoting effect. Interestingly, another study reproduced the Lac-Phe-induced hypophagia in chow-fed lean mice using the same i.p. dose (50 mg $kg^{-1}$), suggesting that the anorexigenic efficacy of Lac-Phe does not depend on nutritional state (61).

Importantly, behavioral analyses ruled out nonspecific or aversive effects. In kaolin consumption and conditioned flavor avoidance assays, two established paradigms for detecting illness-induced malaise, Lac-Phe-treated mice displayed responses comparable to those of controls, suggesting the absence of overt aversive reactions (61). Similarly, locomotor and anxiety-related behaviors assessed by the open field and elevated plus maze tests showed no differences between Lac-Phe-treated and vehicle-treated groups. Additional evaluation in the forced swim and sucrose preference paradigms revealed no evidence of depressive-like behaviors (61). Together, these findings indicate that Lac-Phe does not impair general affective state or induce negative motivational biases. Equally important, Lac-Phe administration in mice did not alter circulating concentrations of classical appetite-regulating hormones such as leptin and ghrelin (58), underscoring that its effects are not mediated through traditional endocrine regulators of energy homeostasis.

In addition, chronic i.p. injection of Lac-Phe (50 mg $kg^{-1}$ $day^{-1}$ for 10 days) in DIO mice elicited sustained reductions in cumulative food intake, body weight, and adiposity while simultaneously improving glucose homeostasis (58). These metabolic benefits occurred without adverse effects on the weights of other major organs, highlighting Lac-Phe's favorable safety profile in preclinical models.

The route of administration proved to be a critical determinant of efficacy. Oral delivery of Lac-Phe in mice failed to reduce food intake or body weight; this failure was most likely due to rapid degradation of the labile peptide bond within the gastrointestinal tract (58). This limitation underscores the need for alternative delivery strategies, such as chemical stabilization, prodrug development, or microbial engineering, to enable translational applications. Furthermore, administration of the precursors lactate or phenylalanine had no effect on feeding or adiposity, demonstrating that the intact conjugate structure of Lac-Phe is essential for biological activity (58).

Evidence from human studies further supports the physiological relevance of Lac-Phe. In an 8-week supervised training program, individuals with higher postexercise Lac-Phe responses experienced greater reductions in abdominal subcutaneous fat and, to a lesser extent, visceral fat (37). These findings extend rodent data to humans, suggesting that Lac-Phe may serve as a mediator of exercise-induced metabolic improvements. Moreover, novel exercise modalities such as blood flow restriction (BFR) training provide additional insights. By partially restricting venous return during muscle contraction, BFR induces local hypoxia and ischemia, thereby enhancing lactate accumulation (10, 56, 57, 95). Given that Lac-Phe biosynthesis requires lactate and phenylalanine, Li and colleagues (55) demonstrated that combining moderate-intensity continuous exercise with BFR significantly elevated Lac-Phe secretion in obese adults. Intriguingly, this rise was associated with appetite suppression and accompanied by increases in ghrelin levels (55), highlighting the complex interplay between Lac-Phe and classical hormonal regulators of hunger.

Collectively, these findings establish Lac-Phe as a physiologically relevant, exercise-inducible metabolite that exerts anorexigenic effects independent of nutritional state. Furthermore, Lac-Phe reduces adiposity and improves glucose tolerance without inducing aversive, anxiogenic, or depressive behavioral responses, supporting its therapeutic potential (**Figure 1**). Identifying the cellular and molecular mediators of Lac-Phe action in the brain will be a critical next step. Such work not only will clarify the precise pathways through which Lac-Phe influences feeding circuits but also may open new avenues for pharmacological strategies aimed at replicating the metabolic benefits of exercise in populations unable to achieve adequate physical activity (63, 112).

### 3.2. Lac-Phe Mediates the Body Weight–Lowering Effects of Metformin

Metformin, prescribed to more than 150 million individuals worldwide, has long been recognized as the keystone of therapy for type 2 diabetes (86). Its primary clinical benefit lies in lowering blood glucose levels and improving insulin sensitivity, thereby reducing both microvascular and macrovascular complications (20, 23, 24, 50, 51, 82). Beyond glycemic control, clinicians have consistently noted a modest but reproducible reduction in body weight among patients treated with metformin (3, 20, 47). This observation is particularly notable given that many other antidiabetic drugs promote weight gain (54). The weight-lowering effect of metformin is thought to derive largely from a reduction in energy intake, rather than alterations in nutrient absorption or changes in basal metabolic rate (51, 116). Although this effect has been consistently demonstrated, the underlying molecular and physiological mechanisms responsible for this anorexigenic effect remain incompletely understood and have been the subject of ongoing debate for decades (45, 46).

Recent advances have identified Lac-Phe as a promising mediator of metformin's metabolic actions, thereby providing a new framework for interpreting these long-standing clinical observations. In 2024, two independent groups, Xiao et al. (113) and Scott et al. (90), concurrently reported that metformin robustly enhances Lac-Phe production in both mice and humans, revealing a previously unrecognized link between pharmacological glucose regulation and exercise-induced metabolic signaling. Specifically, Xiao et al. (113) found that metformin treatment in mice

significantly elevated circulating Lac-Phe concentrations without concomitant increases in blood lactate, indicating a mechanism distinct from the lactate-driven surge observed during exercise. Parallel human studies confirmed that individuals receiving metformin consistently exhibited higher Lac-Phe levels compared with untreated controls (113). Meanwhile, Scott et al. (90) showed that initiation of metformin treatment in newly diagnosed type 2 diabetes patients was associated with a significant rise in circulating Lac-Phe, whereas patients who did not receive metformin displayed no such change. Moreover, clinical metadata analyses identified metformin therapy as the single strongest predictor of serum Lac-Phe levels, surpassing both disease duration and body mass index (90). These findings underscore that metformin is not merely correlated with, but is likely causal in driving, Lac-Phe elevation in vivo. In addition, after 12 weeks of continuous metformin therapy, patients exhibited robust and sustained increases in circulating Lac-Phe (90). Strikingly, even a single oral dose was sufficient to elicit rapid Lac-Phe elevation, with metabolomic profiling over 36 h showing that Lac-Phe rose in parallel with maximal plasma metformin concentrations (90). Overall, these convergent data from acute and chronic interventions provide compelling evidence that Lac-Phe induction is a consistent and reproducible feature of metformin pharmacology (97).

During sprint exercise, Lac-Phe biosynthesis is driven by the mass action of elevated extracellular lactate released from skeletal muscle (113). By contrast, metformin does not trigger a robust increase in circulating lactate, suggesting that Lac-Phe formation is fueled by a distinct intracellular pool (113). Biochemical studies in primary macrophage cells indicate that metformin promotes Lac-Phe synthesis through inhibition of mitochondrial complex I, which enhances glycolytic flux and increases intracellular lactate availability for condensation with phenylalanine (113). Notably, gut-specific CNDP2-KO mice were completely resistant to metformin-induced increases in plasma Lac-Phe, establishing the intestine, particularly epithelial cells, as the principal site of both basal and metformin-inducible Lac-Phe production (113).

In addition, CNDP2-KO mice displayed blunted weight loss in response to metformin compared with wild-type controls, implicating CNDP2-dependent Lac-Phe production as a key determinant of the drug's anorexigenic effects (113). However, it is not yet clear whether gut-specific CNDP2 deletion fully abolishes metformin's weight-lowering actions or whether additional parallel pathways provide partial compensation. Moreover, while metformin has been reported to influence several peptide hormones involved in appetite regulation, including growth differentiation factor 15 (GDF15), glucagon-like peptide-1 (GLP-1), and peptide YY, posttreatment levels of these factors did not differ between wild-type and CNDP2-KO mice (113). Thus, the attenuated response in CNDP2-deficient animals cannot be attributed to differences in these established hormonal signals. For human data, mediation analyses in patient cohorts demonstrated that Lac-Phe acts as a downstream effector of metformin-induced weight reduction, further strengthening that Lac-Phe contributes meaningfully to metformin's antiobesity action (113).

Collectively, these findings place Lac-Phe at the intersection of exercise physiology and pharmacology. Whereas exercise-induced Lac-Phe arises from muscle-derived lactate, metformin-induced Lac-Phe originates primarily in the gut, underscoring that different upstream pathways can converge on the same metabolite to produce similar anorexigenic outcomes (**Figure 1**). This insight not only explains, at least in part, metformin's weight-lowering effects but also highlights Lac-Phe as a point of convergence between lifestyle interventions and pharmacological therapy.

## 4. Lac-Phe REGULATION OF FEEDING CIRCUITS

Despite the promising antiobesity effects of Lac-Phe, the precise mechanisms by which it regulates food intake remain incompletely defined (97). Nonetheless, accumulating evidence

strongly implies the CNS as a primary site of action, with convergent data from pharmacological, neuroanatomical, electrophysiological, and functional approaches (61).

### 4.1. Lac-Phe Induces Hypophagia by Inhibiting Agouti-Related Peptide Neurons

Pharmacological studies provided the first critical insights. Intracerebroventricular (i.c.v.) administration of Lac-Phe robustly reduced food intake in both chow-fed and high-fat diet–fed mice, demonstrating that direct delivery of Lac-Phe into the brain is sufficient to elicit hypophagia (61). Following i.p. injection in mice, Lac-Phe induced robust c-Fos expression in the nucleus tractus solitarius (NTS) and the paraventricular nucleus of the hypothalamus (PVH), two neuronal hubs that integrate peripheral and visceral signals to regulate appetite (61). Given that c-Fos is a well-established immediate early gene marker of neuronal activation, its induction in these regions provides indirect but compelling evidence that peripheral Lac-Phe crosses into the CNS and activates specific neural circuits.

Chemogenetics, most commonly implemented using DREADDs (designer receptors exclusively activated by designer drugs), enables selective activation or silencing of genetically defined neurons in freely moving mice through administration of inert ligands (29, 122, 124). This approach has become a powerful tool for establishing causal links between specific neural populations and behavioral outputs, such as feeding and energy balance. Applying this technique, chemogenetic inhibition of NTS neurons in freely moving mice failed to abolish Lac-Phe-induced hypophagia (61). These results indicate that, although NTS neurons are activated by Lac-Phe administration, they are not essential mediators of its anorexigenic effects. By contrast, silencing PVH neurons markedly attenuated Lac-Phe-induced hypophagia, establishing the PVH as a critical node linking Lac-Phe to reduced feeding (61). This distinction underscores the importance of functional dissection in clarifying which neural activations are epiphenomenal versus causally relevant.

Electrophysiological and circuit-mapping studies provided deeper mechanistic resolution. Electrophysiological recording, particularly whole-cell patch-clamp recording, provides a high-resolution approach for directly assessing neuronal excitability and synaptic activity in brain slices, thereby yielding mechanistic insight into how neural circuits are functionally regulated (7, 30, 36). Using brain slice recording, direct Lac-Phe application did not alter intrinsic excitability of PVH neurons, indicating that PVH activation occurs indirectly, most likely through upstream synaptic inputs (61). Trans-synaptic tracing in mice subsequently identified Agouti-related peptide (AgRP) neurons in the ARH as a direct presynaptic input to Lac-Phe-responsive PVH neurons (61). This finding places AgRP neurons, long recognized as key regulators of energy balance, at the center of Lac-Phe's action. Supporting this, i.p. injection of Lac-Phe in mice elevated concentrations not only in circulation but also within the ARH, confirming that peripheral Lac-Phe can access this critical hypothalamic region (61).

AgRP neurons represent a well-characterized orexigenic population whose inhibition is consistently associated with reduced food intake (107). These neurons release AgRP, neuropeptide Y, and GABA (γ-aminobutyric acid), all of which regulate feeding and modulate energy homeostasis (17, 22, 31, 49, 74, 101, 110). Multiple independent lines of evidence demonstrate that Lac-Phe reduces feeding by inhibiting the activity of AgRP neurons. First, brain slice recording demonstrated that Lac-Phe hyperpolarized AgRP neurons and reduced their firing frequency, effects that persisted even in the presence of synaptic blockers, thereby indicating a direct inhibitory mechanism (61). Second, i.p. injection of Lac-Phe in mice suppressed c-Fos expression in AgRP neurons, and in vivo fiber photometry confirmed marked reductions in AgRP activity in freely moving mice (61). Third, ablation of AgRP neurons abolished Lac-Phe's anorexigenic effect,

whereas chemogenetic activation of AgRP neurons in freely moving mice prevented Lac-Phe or exercise from reducing food intake (61).

Collectively, these findings highlight Lac-Phe as a key molecular mediator that bridges metabolic state and hypothalamic control of appetite. By acting directly on AgRP neurons and indirectly on PVH neurons, Lac-Phe establishes a mechanistic link between peripheral exercise- and nutrient-derived signals and central neural substrates of feeding (**Figure 2**).

## 4.2. Role of $K_{ATP}$ Channels in Lac-Phe Action

The ionic mechanisms underlying Lac-Phe-mediated inhibition of AgRP neurons have received growing attention, as understanding these downstream processes is essential for linking metabolite signaling to neuronal excitability. Among the candidate mechanisms, ATP-sensitive potassium ($K_{ATP}$) channels emerged as particularly strong contenders, given their well-established role as metabolic sensors that couple intracellular energy status to neural activity regulation (64, 65, 84). These channels, expressed at high levels in AgRP neurons, integrate hormonal and nutrient signals such as insulin, leptin, and glucose to fine-tune neuronal activity in accordance with systemic energy demands (34, 104).

Electrophysiological studies have provided compelling evidence implicating $K_{ATP}$ channels in Lac-Phe action. Brain slice recording revealed that Lac-Phe significantly enhanced $K_{ATP}$ channel–mediated currents in AgRP neurons, resulting in hyperpolarization and reduced firing activity (61). These effects were completely abolished by tolbutamide, a selective $K_{ATP}$ channel inhibitor, demonstrating that Lac-Phe suppresses AgRP neuronal activity through a $K_{ATP}$-dependent mechanism (61).

In vivo manipulations provided further support for this mechanism. Central administration of tolbutamide through i.c.v. infusion in mice significantly attenuated Lac-Phe-induced hypophagia, directly linking $K_{ATP}$ activity to behavioral outcomes (61). Moreover, genetic ablation of $K_{ATP}$ channels specifically in AgRP neurons blunted the anorexigenic effect of Lac-Phe in mice, confirming that intact $K_{ATP}$ signaling is indispensable for Lac-Phe's regulation of feeding behavior (61). Collectively, these findings demonstrate that Lac-Phe engages a classical nutrient-sensing pathway in the brain, situating its action within a well-characterized framework of metabolic neurobiology.

Despite these important advances, several critical questions remain unresolved (61). For example, the transport processes by which peripheral Lac-Phe gains access to the ARH remain unclear. Whether specific transporters, such as members of the SLC family recently implicated in Lac-Phe efflux, also facilitate Lac-Phe entry into central circuits is not yet clear. Furthermore, while $K_{ATP}$ channels represent a necessary mediator of Lac-Phe-induced hypophagia, it remains possible that Lac-Phe also modulates additional ionic or synaptic pathways that contribute to its net effects on AgRP neuron activity. The relationship between Lac-Phe and pharmacological interventions further illustrates the complexity of this signaling axis. As discussed above, metformin, one of the most widely prescribed drugs for type 2 diabetes, has been identified as a robust inducer of Lac-Phe in both mice and humans (90, 113). This raises the intriguing hypothesis that part of metformin's anorexigenic effect is mediated through Lac-Phe-induced inhibition of AgRP neurons. However, whether Lac-Phe represents the primary mediator of metformin's effects or instead functions in parallel with other metformin-regulated pathways such as GDF15 signaling remains to be clarified.

Moving forward, future research should determine how Lac-Phe bioavailability and neuronal actions are influenced by metabolic status and other physiological conditions. Such studies will provide a more complete understanding of Lac-Phe's physiological roles and help define its therapeutic potential as a molecular link between exercise, brain function, and metabolic health.

## 5. Lac-Phe AS A BIOMARKER FOR EARLY DISEASE DETECTION

Recent advances have highlighted the emerging importance of Lac-Phe as both a potential biomarker and a therapeutic target across diverse pathological conditions (69). The concept of using Lac-Phe as a biomarker is particularly compelling for early disease detection and the development of personalized interventions, especially given the correlations observed between its circulating levels and disease onset or severity (69). Nevertheless, it is critical to recognize that most current studies have identified associative rather than causal relationships. Clarifying whether Lac-Phe actively contributes to disease pathogenesis or instead reflects altered metabolic states remains a central challenge for the field (69).

Sepsis is a life-threatening syndrome marked by dysregulated host inflammatory responses to infection, leading to multiorgan dysfunction and high mortality (94). Substantial evidence points to mitochondrial dysfunction as a central feature of sepsis pathophysiology (4, 93), but quantifying mitochondrial failure in patients has been technically difficult. Building on prior observations that Lac-Phe is markedly elevated in genetic mitochondrial disorders and correlates with both disease severity and heteroplasmy (91, 111), Rogers and colleagues (85) reported that circulating Lac-Phe levels were significantly increased in patients with septic shock compared with other critically ill groups. These findings suggest that Lac-Phe may serve as a proxy marker of mitochondrial dysfunction and, more importantly, as a predictor of mortality in septic shock (85). Thus, Lac-Phe has the potential to refine risk stratification in intensive care settings and to guide therapeutic decisions while also offering new avenues for probing mitochondrial pathophysiology in sepsis.

Glaucoma, the world's second leading cause of blindness, is characterized by progressive degeneration of retinal ganglion cells, ultimately leading to irreversible vision loss (99). Current therapies for primary open-angle glaucoma (POAG) focus largely on lowering intraocular pressure (IOP) (96). However, a subset of patients continue to progress despite adequate IOP control, underscoring the need for biomarkers that can predict disease risk and progression (1, 53). Tang and colleagues (96) reported that plasma Lac-Phe levels were significantly reduced in patients with POAG and proposed Lac-Phe as a promising biomarker candidate on the basis of its high area-under-the-curve values and clinical relevance. While these findings suggest potential utility, the relatively small sample size and limited geographic diversity of the study population constrain their generalizability (96). Larger, multiethnic cohort studies will be required to validate Lac-Phe as a robust biomarker for glaucoma, and mechanistic studies are needed to determine whether Lac-Phe plays a causal role in retinal degeneration or functions as a systemic correlation of neurodegenerative processes.

Beyond ocular and infectious diseases, Lac-Phe has also been implicated in inborn errors of metabolism. Phenylketonuria (PKU) is a classical monogenic disorder caused by mutations in phenylalanine hydroxylase, leading to elevated circulating phenylalanine levels and neurocognitive impairments (8, 105). Although early diagnosis via newborn screening and adherence to dietary therapy have greatly improved outcomes, residual neurocognitive and psychiatric complications remain prevalent (105). In 2024, van Wegberg and colleagues (106) reported that Lac-Phe levels were markedly elevated in PKU patients compared with healthy controls and, strikingly, were more strongly associated with cognitive outcomes than was phenylalanine itself, suggesting that Lac-Phe may not simply be a downstream by-product of disrupted amino acid metabolism but may instead represent a mechanistic contributor to PKU-associated neurocognitive pathology. If validated, this finding could reshape the biomarker landscape in PKU by positioning Lac-Phe as both a predictor of cognitive outcomes and a potential therapeutic target.

Collectively, these diverse lines of evidence position Lac-Phe as a metabolite of substantial biomedical interest. Its associations with conditions such as sepsis, glaucoma, and PKU highlight

its promise as a clinically relevant biomarker (**Figure 3**). At the same time, the field must address the critical question of whether Lac-Phe actively contributes to disease pathogenesis or primarily reflects shifts in metabolic state. Addressing this distinction will be essential for determining whether Lac-Phe can be harnessed merely as a biomarker for diagnosis and prognosis or also as a therapeutic target to modify disease trajectories. Future work should therefore integrate metabolomic, genetic, and mechanistic studies in both animal models and human cohorts to clarify the causal role of Lac-Phe and to evaluate its translational potential across multiple disease contexts.

## 6. BHB-Phe: A CNDP2-DERIVED STRUCTURAL ANALOG OF Lac-Phe

Mammals have evolved intricate nutrient-responsive pathways that dynamically couple external energy availability to the maintenance of internal metabolic homeostasis (68). Central to these pathways are fluctuations in cellular energy metabolites, which serve not only as metabolic fuels but also as signaling effectors that convey information about systemic energy status. A well-studied example is β-hydroxybutyrate (BHB), a ketone body whose circulating levels increase during periods of carbohydrate scarcity, such as starvation, intermittent fasting, or adherence to a ketogenic diet (70, 78). Historically recognized as an oxidative substrate for ATP generation in metabolically active tissues like the brain and heart, BHB is now appreciated as a versatile signaling molecule (68). It activates G protein–coupled receptors (43, 79), mediates posttranslational protein modifications (114), and inhibits histone deacetylases (92), thereby exerting broad regulatory control over gene expression, cellular metabolism, and physiological function.

Building upon this paradigm, recent research has revealed a direct enzymatic link between BHB and Lac-Phe metabolism, providing a new example of how intermediary metabolites are integrated into signaling networks (68). In 2025, Moya-Garzon and colleagues (68) demonstrated that CNDP2, the cytosolic enzyme responsible for Lac-Phe biosynthesis, also catalyzes the conjugation of BHB to free amino acids in HEK293T cells and in mouse tissues. This enzymatic activity yields a novel class of compounds, BHB–amino acid conjugates, which are endogenously detectable in both mouse and human plasma, are inducible under conditions of ketosis, and are subject to genetic regulation (68). Among these conjugates, *N*-β-hydroxybutyryl phenylalanine (BHB-Phe) emerged as the most abundant, representing a structural analog of Lac-Phe (68). Importantly, comparative biochemical assays in HEK293T cells revealed that CNDP2-dependent Lac-Phe synthesis proceeds more rapidly than BHB-Phe synthesis under saturating substrate concentrations, while active-site mutations in CNDP2 impair the generation of both metabolites (68). Tissue distribution studies further identified the kidney and gut as the primary sites of BHB-Phe biosynthetic activity, which was largely abolished in CNDP2-KO mice, firmly establishing CNDP2 as the principal enzyme mediating BHB-Phe production in vivo (68).

Given the structural similarity between Lac-Phe and BHB-Phe as well as their shared CNDP2-dependent biosynthetic pathway, BHB-Phe has been hypothesized to exert comparable physiological effects on energy balance. In DIO mice, acute i.p. injection of BHB-Phe (50 mg $kg^{-1}$) significantly reduced food intake without altering locomotor activity, oxygen consumption, or carbon dioxide production (68). Notably, circulating concentrations of classical appetite-regulating hormones, including ghrelin, leptin, and GDF15, remained unchanged after administration, suggesting that the anorexigenic effects of BHB-Phe are not mediated through these hormonal pathways (68). Furthermore, chronic i.p. injection of BHB-Phe (50 mg $kg^{-1}$ $day^{-1}$ for 14 days) produced a sustained suppression of daily food intake and attenuated body weight gain in DIO mice, thereby reinforcing its potential relevance to long-term energy balance regulation (68). These results mirror the effects observed with Lac-Phe but also raise the possibility of distinct mechanisms.

To investigate the neural substrates engaged by BHB-Phe, Moya-Garzon and colleagues (68) employed activity-dependent labeling (TRAP, or targeted recombination in active populations) (18). TRAP is a genetic strategy that permanently labels neurons activated during a defined stimulus window (32). By linking immediate early gene promoters to inducible Cre recombinase, TRAP enables mapping and manipulation of functionally relevant neuronal ensembles in freely moving animals (32, 52). The i.p. injection of either BHB-Phe or Lac-Phe in mice activated neuronal ensembles across multiple brain regions implicated in feeding regulation, including the PVH, suprachiasmatic nucleus, dorsomedial hypothalamus, ventromedial hypothalamus, ARH, lateral hypothalamus, lateral parabrachial nucleus, and NTS (68). While both metabolites engaged widespread circuits, only a small subset of neurons was coactivated, indicating that BHB-Phe and Lac-Phe converge on partially overlapping but largely distinct neural pathways (68). This observation suggests that structurally related conjugates such as Lac-Phe and BHB-Phe, produced by CNDP2, may diversify signaling outputs, thereby providing a richer repertoire of metabolic cues to shape feeding and energy homeostasis.

Overall, the identification of BHB-Phe as a Lac-Phe-related metabolite expands the scope of CNDP2-derived signaling conjugates and implicates them in the regulation of feeding behavior. At the same time, critical questions remain unresolved (68). The molecular targets of BHB-Phe have not been identified, leaving open whether its effects are mediated by G protein–coupled receptors, ion channels, or alternative mechanisms. Future research aimed at identifying BHB-Phe's receptor or receptors, delineating its downstream signaling pathways, and clarifying the specific neuronal populations involved will be essential to integrating this metabolite into the broader framework of nutrient-derived signaling. Such work will ultimately determine whether BHB-Phe, like Lac-Phe, can be leveraged as a therapeutic target to modulate appetite and energy balance and whether CNDP2-derived conjugates represent a generalizable paradigm for metabolite-driven regulation of brain function and behavior.

## 7. EMERGING PHYSIOLOGICAL AND THERAPEUTIC FUNCTIONS OF Lac-Phe

Lac-Phe, an exercise-inducible metabolite, has recently emerged as a multifunctional bioactive compound with wide-ranging physiological and therapeutic implications. Beyond its now well-established role in appetite suppression, accumulating evidence suggests that Lac-Phe also contributes to tissue regeneration, immune regulation, and metabolic health (62, 117, 118). Together, these findings position Lac-Phe as a promising candidate for both mechanistic exploration and translational application.

One of the most intriguing observations is that Lac-Phe appears to couple regenerative processes with metabolic regulation. Recent studies report that Lac-Phe promotes osteochondral regeneration while simultaneously inhibiting adipogenesis in bone marrow mesenchymal stem cells, thereby offering a dual benefit: restoration of joint integrity alongside suppression of excess fat accumulation (62). Such properties highlight Lac-Phe's potential utility in the treatment of osteochondral defects, a condition for which effective regenerative strategies remain limited (62). By simultaneously targeting musculoskeletal and metabolic pathways, Lac-Phe provides a conceptual framework for therapies that address the intersection of structural repair and energy balance.

In addition to having regenerative functions, Lac-Phe exerts immunomodulatory effects. Inflammatory bowel disease (IBD), which encompasses ulcerative colitis and Crohn's disease, is a chronic, relapsing disorder characterized by dysregulated innate and adaptive immune responses (5, 11, 26, 102). Recent work by Yu et al. (118) demonstrated that Lac-Phe attenuates experimental colitis in mice by inhibiting NF-κB signaling, suppressing M1 macrophage activation, and

reducing production of proinflammatory cytokines. Notably, dietary supplementation with phenylalanine partially enhanced Lac-Phe production, suggesting that both Lac-Phe and its precursors may hold therapeutic potential in IBD (118). This finding introduces the possibility of leveraging diet–metabolite interactions as a novel axis for immune modulation.

Lac-Phe has also been implicated in CNS repair. Spinal cord injury (SCI) is a devastating neurological disorder that often results in permanent impairments due to limited regenerative capacity (25). Following SCI, pathological cascades involving neuroinflammation, oxidative stress, and metabolic dysfunction exacerbate tissue damage and hinder recovery (19). Ying et al. showed that Lac-Phe administration significantly enhanced functional recovery in mice with SCI, an effect accompanied by improved lipid metabolism and reduced activation of proinflammatory microglia and macrophages (117). These results not only provide a mechanistic link between Lac-Phe and CNS repair but also underscore the broader therapeutic promise of targeting immune–metabolic interactions in neurodegeneration (117).

Overall, these diverse lines of evidence underscore the multifaceted nature of Lac-Phe biology. By linking appetite suppression, tissue regeneration, immune modulation, and neurorepair, Lac-Phe illustrates how exercise-inducible metabolites can extend beyond traditional metabolic signaling to shape both health and disease (**Figure 3**). At the same time, ongoing pharmacological and methodological innovations highlight the opportunities for harnessing Lac-Phe as a therapeutic agent in clinical practice.

## 8. FUTURE DIRECTIONS

In addition to having well-established benefits for cardiovascular and metabolic health, regular physical activity is increasingly recognized as a foundation of overall physiological well-being, contributing to the prevention of pregnancy-related disorders and the mitigation of age-associated cognitive decline (71, 83, 121). During pregnancy, consistent exercise is associated with multiple favorable outcomes, including reduced incidence of gestational diabetes and hypertensive disorders, lower rates of operative delivery, and diminished postpartum weight retention and depression (12–16). Given that Lac-Phe is among the most robustly induced metabolites following exercise, it is intriguing to consider whether Lac-Phe contributes to these physiological benefits. Moreover, physical exercise has long been known to attenuate cognitive decline with aging by promoting neurogenesis, angiogenesis, and synaptic plasticity while reducing inflammation and oxidative damage (21, 80, 88). Whether Lac-Phe functions as a key mediator of these neuroprotective and systemic effects remains a compelling question for future investigation.

Although CNDP2 is recognized as the principal enzyme responsible for Lac-Phe biosynthesis, CNDP2 alone cannot fully account for Lac-Phe production. CNDP2-KO mice retain partial Lac-Phe levels (63), indicating that alternative biosynthetic routes exist. Thus, future research should aim to identify additional enzymes or pathways that contribute to Lac-Phe production under conditions of CNDP2 deficiency. Clarifying these mechanisms will be essential for a comprehensive understanding of Lac-Phe biology and may reveal compensatory networks that sustain Lac-Phe availability.

Emerging evidence also implicates Lac-Phe in mediating the metabolic actions of metformin, particularly its antiobesity effects. However, the extent to which Lac-Phe is necessary and sufficient for the full range of metformin's benefits, including its influence on glucose regulation, remains to be determined (113). Moreover, whether Lac-Phe interacts synergistically or antagonistically with other metformin-induced signals and whether its production varies across intestinal regions or specific cell types represent important areas for exploration (113). Addressing these questions could guide the design of therapeutic strategies that enhance Lac-Phe synthesis or mimic its downstream effects to optimize the cardiometabolic efficacy of metformin.

Despite growing enthusiasm for the potential therapeutic use of Lac-Phe, key pharmacological challenges persist. Notably, only i.p. injection of Lac-Phe reliably suppresses feeding in obese mice, whereas oral administration fails to reduce food intake or body weight (58). This limitation is likely attributable to degradation of the peptide bond within the digestive tract. Since oral delivery is more practical and clinically feasible than injection, novel strategies to improve bioavailability are under active investigation. Ottosen et al. (76) recently developed a low-molecular-weight Lac-Phe ester that can be administered orally and effectively elevates circulating Lac-Phe concentrations in rats. In parallel, Kim et al. (42) identified Lac-Phe in kimchi produced by lactic acid bacteria strains, raising the intriguing possibility that fermented foods may serve as natural dietary sources of Lac-Phe with potential metabolic benefits.

Collectively, these avenues, ranging from mechanistic elucidation of Lac-Phe biosynthesis and signaling to the translational development of pharmacological and nutritional interventions, highlight the expanding therapeutic landscape for this exercise-induced metabolite. Continued investigation into the physiological roles, biosynthetic diversity, and clinical applications of Lac-Phe not only will deepen understanding of exercise biology but may also reveal new strategies for combating obesity, metabolic disease, and age-related functional decline.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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