Review

From Network to Code: The Calci-Keto-Inflammatory Language of Metabolic Adaptation

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From Network to Code: The Calci-Keto-Inflammatory Language of Metabolic Adaptation

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Abstract

The transition from energy sufficiency to deficiency triggers complex metabolic and immune adaptations that have traditionally been viewed through a pathological perspective. This review introduces the Keto-Inflammatory Network (KIN), a novel framework positioning ketosis as an evolutionarily conserved adaptive response rather than metabolic dysfunction. The KIN integrates β-hydroxybutyrate (BHB) signaling with immune modulation, epigenetic regulation, circadian rhythms, and microbiota interactions. Through mechanisms including NLRP3 inflammasome inhibition, HDAC-mediated epigenetic modifications, and HCAR2 receptor activation, ketone bodies orchestrate anti-inflammatory responses while maintaining metabolic flexibility. The framework encompasses calcium-ketone integration through the Calci-Keto-Inflammatory Code (CKOC), temporal regulation via the Ketoinflammatory Clock, and trans-kingdom signaling through microbiota interactions. In dairy cattle, this perspective reframes periparturient ketosis as potentially adaptive, supporting lactogenesis and immune tolerance during reproductive transition. The CKOC paradigm suggests therapeutic applications leveraging ketone signaling for inflammatory diseases, autoimmune conditions, and metabolic disorders while challenging traditional ketosis management strategies. This systems-level understanding opens new avenues for precision interventions that work with, rather than against, ancient adaptive mechanisms refined through evolution.

Keywords: ketosis; inflammation; immunometabolism; β-hydroxybutyrate; dairy cattle; epigenetics; microbiota; calcium signaling; circadian rhythms; adaptive immunity

1. Introduction

The evolutionary transition from unicellular to multicellular life necessitated the development of sophisticated communication systems capable of integrating metabolism, immunity, and environmental sensing into coherent organismal responses [1,2]. Among the most ancient and conserved of these biological information processing systems is the capacity for ketogenesis - the hepatic production of ketone bodies from fatty acid precursors during periods of altered energy availability [3,4]. While ketosis has been traditionally conceptualized through the reductionist framework of metabolic compensation for glucose deficiency - a pathological state requiring suppression - recent systems-level investigations have revealed ketone bodies, particularly β-hydroxybutyrate (BHB), as pleiotropic signaling molecules that encode complex physiological information and orchestrate adaptive programs extending far beyond simple energy provision [5–7].

The remarkable conservation of ketogenic pathways across vertebrate evolution -from arctic mammals enduring months of food scarcity to migratory birds completing transcontinental flights without feeding - suggests fundamental adaptive significance that transcends the pathological framing dominant in medical and veterinary literature [1,8]. This evolutionary persistence implies that ketone bodies function as carriers of survival-relevant information about nutritional status, immune challenges, temporal phase, and environmental conditions - information that must be reliably encoded, transmitted, and decoded to generate appropriate adaptive responses [5,7]. In humans, physiological ketosis occurs naturally during multiple life stages and conditions including

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prolonged fasting, intensive exercise, pregnancy, lactation, and early postnatal development, each representing distinct informational contexts requiring coordinated metabolic-immune adjustments [3,9]. Yet conventional medical paradigms have systematically pathologized these natural states, largely based on extrapolation from extreme pathological conditions such as diabetic ketoacidosis - a conceptual error equivalent to condemning fever because hyperthermia is dangerous [10,11].

The periparturient dairy cow represents perhaps the most intensive natural model of integrated metabolic-immune information processing, where the physiological demands of late pregnancy, parturition, and early lactation initiate coordinated systemic transitions involving massive energy mobilization, dramatic hormonal restructuring, immune system modulation, and fundamental alterations in microbial ecology [12,13]. During this critical transition period, plasma BHB concentrations can increase 5- to 10-fold within days of calving, coinciding with profound alterations in circulating immune cell populations, systemic inflammatory mediator profiles, and calcium homeostasis [13–15]. Veterinary medicine has conventionally treated this ketone elevation as primary pathology requiring aggressive suppression, despite limited evidence that such interventions improve long-term outcomes and growing recognition that early ketone elevation may represent adaptive programming [14,15]. The temporal sequence and pattern of these metabolic changes - not merely their magnitude - appears to encode diagnostic information that distinguishes adaptive from pathological trajectories, suggesting that biological systems "read" complex metabolic signatures to coordinate appropriate responses [14,15].

Recent paradigm-shifting discoveries in molecular immunology and metabolic biochemistry have fundamentally challenged reductionist conceptual boundaries separating metabolism and immune function [14,16]. The recognition that immune cells undergo systematic metabolic reprogramming during activation states, that small molecule metabolites function as potent cellular signaling mediators with context-dependent meanings, and that mitochondria serve as critical immune sensing organelles has created entirely new theoretical frameworks for understanding health, disease, and therapeutic intervention [16,17]. These discoveries reveal that metabolites are not merely biochemical intermediates in linear pathways but information carriers - molecular symbols in biological languages that enable cellular communication and coordinate distributed decision-making across tissues and organ systems. Yet these insights have been slow to penetrate clinical thinking about ketosis, which remains dominated by threshold-based pathology models focused on metabolite normalization rather than understanding the biological information encoded in metabolic patterns.

This comprehensive review challenges the reductionist pathological paradigm by introducing a systems-level framework for understanding ketone metabolism. We begin by characterizing the **Keto-Inflammatory Network (KIN)** - the interconnected system of metabolic, inflammatory, immune, microbial, and circadian pathways through which ketone bodies exert their pleiotropic effects [5–7]. This network perspective reveals connections invisible to reductionist approaches: bidirectional metabolic-immune crosstalk, trans-kingdom signaling between host and microbiome, temporal coordination through circadian mechanisms, and multi-scale integration from molecular interactions to whole-organism physiology.

As our analysis deepens, we recognize that network connectivity alone inadequately captures the sophisticated information processing properties exhibited by this system. The evidence compels us toward a more fundamental reconceptualization: the **Ketoinflammatory Code** - a biological information processing system with true code-like properties including syntax (molecular grammar governing signal patterns), semantics (biological meaning emerging from metabolic signatures), and pragmatics (context-dependent interpretation generating appropriate adaptive responses). This framework positions ketosis not as metabolic failure but as an evolutionarily conserved adaptive program - an encoded biological language through which organisms assess their state, communicate between distributed cellular systems, and coordinate situation-appropriate responses that optimize survival and reproductive success under diverse environmental challenges.

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By progressing from network architecture to code comprehension, we provide both deeper mechanistic insights into how ketone signaling operates and more sophisticated therapeutic strategies that support rather than suppress evolved regulatory intelligence. This review synthesizes evidence from molecular biology, immunology, microbiology, chronobiology, and clinical investigation to demonstrate that ketosis represents sophisticated biological adaptation warranting respect and, when necessary, informed modulation - not reflexive pathologization and suppression. The framework we present directly challenges century-old paradigms while providing practical tools for predictive diagnostics and therapeutic innovation in both human and veterinary medicine.

2. The Reductionist Paradigm - Ketosis as Pathology

The dominant medical and veterinary paradigm characterizing ketosis has been shaped by over a century of observations made primarily within pathological contexts, creating a deeply embedded conceptual framework that views any elevation in ketone body concentrations as inherently problematic and requiring therapeutic intervention [10,18]. This reductionist perspective emerged from early clinical observations of diabetic ketoacidosis, starvation ketosis in malnourished populations, and periparturient ketosis in high-producing dairy cattle, where extreme ketone elevations were consistently associated with morbidity, reduced performance, and increased mortality risk [5,10,19]. Critically, this pathological framing has systematically ignored the possibility that ketone bodies might function as signaling molecules encoding biological information, instead treating them exclusively as toxic metabolic byproducts requiring elimination.

Within the traditional veterinary framework, ketosis in dairy cattle has been conceptualized as a direct consequence of insufficient energy intake relative to the enormous metabolic demands of early lactation milk synthesis (**Figure 1**) [12,20]. This energy deficit model proposes that when dietary energy intake fails to meet the 40-60% increase in energy requirements associated with peak milk production, stored adipose tissue lipids are mobilized through lipolysis, overwhelming hepatic oxidative capacity and resulting in ketone accumulation [20,21]. This framework treats ketone elevation as simple metabolic overflow - analogous to water spilling from an overfilled container - with no consideration that ketone production might be actively regulated to communicate metabolic state to distant tissues. However, recent metabolomics investigations have revealed that the pathophysiology of ketosis is far more complex than simple energy deficiency, with alterations in immune function, inflammatory mediators, and metabolic pathways detectable weeks before clinical disease manifestation [14]. The predictive power of these early metabolic signatures suggests coordinated biological programming rather than passive metabolic failure - a finding fundamentally incompatible with the overflow model [14]. The clinical classification system distinguishing between subclinical ketosis (elevated blood BHB concentrations without overt clinical signs) and clinical ketosis has reinforced the perception that any detectable ketone elevation represents pathological departure from normal physiological function [13,22].

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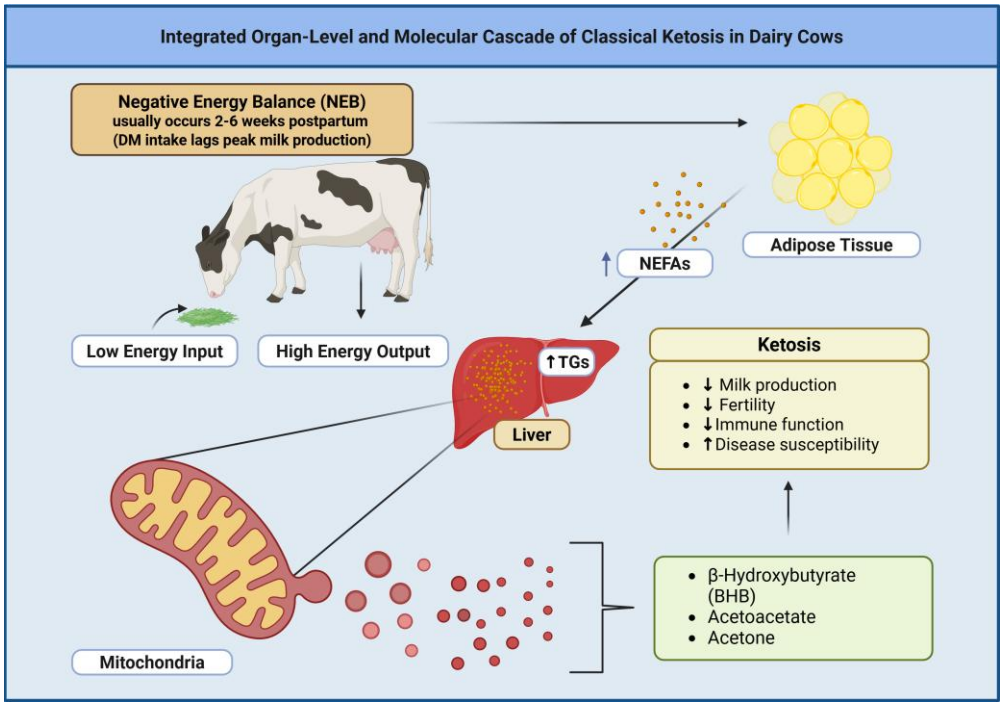

Figure 1. Integrated Organ-Level and Molecular Cascade of Classical Ketosis in Dairy Cows. This diagram illustrates the physiological and metabolic sequence underlying classical ketosis in early-lactation dairy cows. Negative energy balance (NEB) arises when dietary energy intake fails to meet the high demands of milk synthesis during early lactation. In response, adipose tissue mobilizes non-esterified fatty acids (NEFAs) into circulation. The liver takes up these NEFAs, directing them to β-oxidation for energy. When hepatic acetyl-CoA production exceeds the capacity of the tricarboxylic acid (TCA) cycle—mainly due to oxaloacetate depletion from gluconeogenesis—excess acetyl-CoA is diverted to ketogenesis, producing β-hydroxybutyrate (BHB), acetoacetate, and acetone. This overflow leads to subclinical or clinical ketosis, characterized by elevated ketone bodies, reduced milk yield, impaired fertility, suppressed immune function, increased disease susceptibility, and hepatic triglyceride accumulation. [Created in BioRender by Jincheol and Jingyu Choi; (2025) https://BioRender.com/dh9j163].

Epidemiological investigations have consistently documented that 40-60% of periparturient dairy cows experience some degree of ketosis within the first three weeks following calving, with significant economic impacts including direct treatment costs and indirect consequences such as reduced milk yield, impaired reproductive performance, increased susceptibility to infectious diseases, and elevated culling rates [13,23]. Importantly, cows with high somatic cell counts prior to dry-off show increased likelihood of developing ketosis, suggesting interconnected disease processes rather than isolated metabolic dysfunction [24]. These epidemiological associations, while traditionally interpreted as ketosis causing secondary pathologies, are equally consistent with ketosis representing an integrated response to underlying inflammatory or immune dysregulation -a possibility the reductionist paradigm has not adequately explored.

The pathophysiological model underlying traditional ketosis management focuses on the complex endocrine changes accompanying negative energy balance (NEB), including decreased insulin concentrations, elevated glucagon, cortisol, and growth hormone levels that collectively stimulate non-esterified fatty acid (NEFA) mobilization from adipose tissue stores [25,26]. These mobilized fatty acids undergo hepatic β-oxidation through the carnitine palmitoyltransferase system, and when the rate of acetyl-CoA production exceeds the oxidative capacity of the tricarboxylic acid cycle, excess acetyl-CoA is diverted into ketogenesis through the mitochondrial enzymes acetyl-CoA acetyltransferase, 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2), and HMG-CoA lyase [4,27]. While biochemically accurate, this mechanistic description implicitly frames ketogenesis as

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passive consequence of substrate availability rather than actively regulated biological process - ignoring substantial evidence for transcriptional, post-translational, and allosteric regulation of ketogenic enzymes that would enable ketone production to function as controlled signaling rather than uncontrolled overflow.

Traditional therapeutic approaches have emphasized minimizing energy deficits through strategic supplementation with gluconeogenic precursors including propylene glycol and sodium propionate, energy-dense bypass fats that avoid ruminal fermentation, and feed additives such as monensin that improve feed conversion efficiency and propionate production [28,29]. While these approaches have demonstrated efficacy in reducing ketone concentrations and improving short-term clinical outcomes, they fundamentally operate from the assumption that ketosis represents metabolic dysfunction requiring correction rather than adaptive physiology that might serve beneficial biological functions [5,30,31]. More problematically, by focusing exclusively on suppressing ketone production or enhancing clearance, these interventions may inadvertently disrupt biological signaling processes - analogous to treating fever by suppressing thermogenesis without addressing why the organism generated elevated temperature in the first place.

The limitations of this reductionist paradigm have become increasingly apparent as molecular and systems biology reveal sophisticated regulatory networks connecting ketone metabolism with immune function, circadian rhythms, epigenetic regulation, and microbial ecology. The growing recognition that ketone bodies, particularly BHB, function as signaling molecules through G-protein coupled receptors, as histone deacetylase inhibitors influencing gene expression, and as inflammasome regulators modulating immune responses demands fundamental reconceptualization of what ketosis represents biologically [6,35]. The remainder of this review presents an alternative framework - grounded in network analysis and information theory - that repositions ketosis from metabolic pathology to sophisticated adaptive program, with profound implications for both mechanistic understanding and therapeutic strategy.

3. The Keto-Inflammatory Network – A Systems Level Framework

The classical reductionist view of ketosis as passive metabolic overflow fundamentally fails to account for the sophisticated regulatory capabilities and evolutionary conservation characterizing ketone body metabolism across diverse species and physiological contexts [1,16]. Contemporary systems biology approaches reveal that biological networks operate through complex, multi-layered integration systems capable of generating context-appropriate responses that optimize organismal fitness under varying environmental conditions [16,32,33].

The **Keto-Inflammatory Network** (KIN) represents a theoretical framework that reconceptualizes ketosis as a sophisticated, evolutionarily refined biological program in which ketone bodies coordinate metabolic flexibility, immune modulation, stress adaptation, and cellular communication across multiple organ systems (Figure 2) [5–7]. This paradigm shift moves beyond viewing ketones merely as alternative energy substrates or toxic metabolic byproducts to recognizing their roles as regulatory molecules capable of orchestrating complex physiological adaptations that enhance survival and reproductive success under diverse environmental challenges.

3.1. Network Architecture: Molecular Integration and Distributed Processing

The fundamental architecture of the KIN operates through multiple interconnected regulatory nodes that sense, integrate, and respond to diverse physiological signals including energy availability, inflammatory status, microbial ecology, circadian timing, environmental stressors, and developmental stage [16,34]. Central to this architecture is BHB, which serves simultaneously as energy substrate, G-protein coupled receptor ligand (GPR109A/HCAR2), histone deacetylase inhibitor, post-translational modifier (β-hydroxybutyrylation), and inflammasome regulator [6,7,35]. This multiplexed functionality creates potential for coordinated responses across multiple biological systems [35,36].

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The network exhibits architectural features characteristic of evolved biological systems. Redundancy provides fault tolerance through multiple pathways for ketone production (hepatic ketogenesis, astrocytic synthesis) and sensing (receptor-mediated, metabolic, epigenetic). Distributed processing generates emergent behavior from local decisions by hepatocytes, immune cells, neurons, and other cell types, without requiring centralized control. Scale-free topology employs hub nodes such as hepatic ketogenesis and inflammatory mediators to coordinate activity across widely distributed peripheral components [16,32]. These properties enable the network to maintain function despite perturbations and to coordinate responses across vastly different biological scales - from molecular interactions within individual cells to whole-organism behavioral and metabolic adjustments.

Critically, the network generates qualitatively different responses depending on the specific combination of input signals, duration of activation, and physiological context [6,17]. Identical ketone concentrations produce different biological outcomes based on concurrent inflammatory signals, nutritional status, or circadian phase - a functional plasticity that distinguishes the KIN from simple linear metabolic pathways.

3.2. Predictive Metabolomics: Evidence for Coordinated Programming

The systems veterinary medicine approach has provided compelling experimental evidence that ketosis represents coordinated biological programming rather than acute metabolic failure [16]. Multi-platform metabolomics investigations reveal that metabolite signatures can distinguish animals that will develop ketosis from those that will remain healthy weeks before clinical manifestation, achieving prediction accuracies exceeding 90% [93].

The patterns that distinguish pre-ketotic from healthy animals extend far beyond ketone body concentrations themselves, encompassing coordinated alterations in amino acid profiles, lipid species, inflammatory mediators, and microbial metabolites [93]. This multi-metabolite nature demonstrates that the network operates through coordinated changes across multiple metabolic pathways rather than isolated perturbations in ketone production. The predictive power of these signatures - their ability to forecast outcomes weeks in advance - indicates that the biological system undergoes progressive, orchestrated changes rather than sudden metabolic collapse.

Furthermore, immune system alterations frequently precede rather than follow metabolic dysfunction, challenging assumptions that ketosis represents primary metabolic failure with secondary immune consequences [14]. This temporal architecture reveals bidirectional metabolic-immune crosstalk, with the network integrating signals from multiple physiological domains to generate adaptive responses. The recognition that immune status modulates metabolic programming fundamentally distinguishes the KIN framework from traditional views of ketosis as purely metabolic pathology.

3.3. Evolutionary Conservation: Fundamental Biological Importance

The evolutionary conservation of KIN components across mammalian species provides compelling evidence for fundamental biological importance extending beyond species-specific metabolic adaptations [1,2,27]. Comparative genomic analyses reveal high conservation of ketogenic enzymes (HMGCS2, BDH1, ACAT1), ketone-sensing receptors (GPR109A), and downstream signaling pathways from rodents to ruminants to primates, suggesting that core network functionality has been maintained through selective pressure over millions of years of vertebrate evolution [27,37].

Such deep conservation implies that the network solves fundamental survival problems common to diverse species and ecological niches - coordinating distributed cellular responses to fluctuating nutritional availability while simultaneously modulating immune function, maintaining neural activity, and preserving reproductive capacity. The fact that organisms as metabolically divergent as obligate carnivores and herbivores retain similar ketone signaling machinery suggests

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that the network's value lies in its capacity to integrate and communicate organismal state across tissues.

The distribution of ketone-responsive elements across functionally diverse tissues -brain, heart, skeletal muscle, immune cells, kidneys, and adipose tissue - indicates that the network evolved as a whole-organism coordination mechanism [6,35]. This architectural feature ensures that local cellular decisions align with organism-level metabolic and immunological state, enabling coherent adaptive responses to environmental challenges.

3.4. Implications of the Network Framework

The KIN framework fundamentally challenges reductionist paradigms by demonstrating that ketosis involves coordinated multi-system responses rather than isolated metabolic dysfunction. Network analysis reveals sophisticated regulatory architecture with redundancy, distributed processing, and multi-scale integration capabilities that enable adaptive flexibility under diverse physiological conditions. The predictive power of metabolomics signatures and the evolutionary conservation of network components provide compelling evidence that ketone metabolism represents an adaptive biological program rather than compensatory overflow.

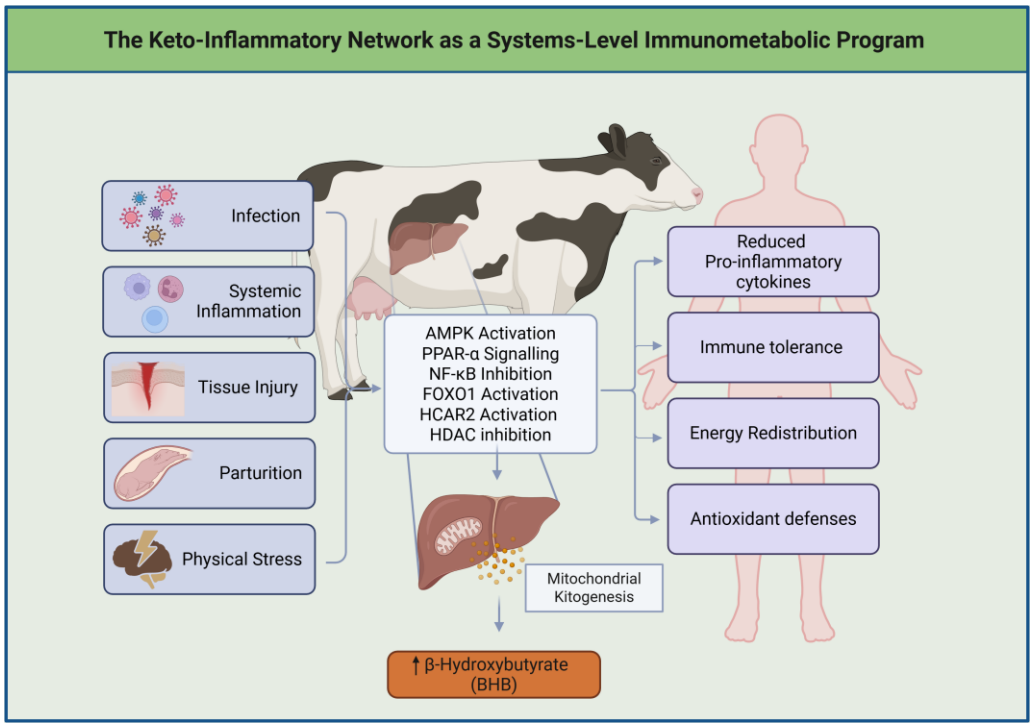

Figure 2. The Keto-Inflammatory Network as a Systems-Level Immunometabolic Program. This diagram illustrates how elevated β-hydroxybutyrate (BHB), generated via hepatic mitochondrial ketogenesis during metabolic stress, acts as a systemic signaling molecule to coordinate immunometabolic adaptation. Physiological stressors - such as infection, systemic inflammation, tissue injury, parturition, and physical stress - trigger hepatic mitochondrial reprograming via AMPK and PPAR-α activation. The resulting increase in circulating BHB modulates several conserved molecular pathways, including HCAR2 activation, HDAC inhibition, FOXO1 activation, and suppression of NF-κB signaling. These pathways converge to reduce pro-inflammatory cytokine production, promote immune tolerance, redistribute metabolic energy, and enhance antioxidant defenses. Collectively, these integrated responses constitute the Keto-Inflammatory Network (KIN) - a conserved system that links metabolic stress to inflammation resolution and homeostatic rebalancing. [Created in BioRender by Jincheol and Jingyu Choi; (2025) https://BioRender.com/968gf5a].

This network perspective provides the foundation for understanding the molecular mechanisms, regulatory interactions, and physiological functions detailed in subsequent sections.

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The KIN framework enables investigation of specific network components - including calcium integration, circadian coordination, microbial interactions, and epigenetic programming - that collectively generate the sophisticated adaptive responses observed during physiological and pathological ketosis.

4. Triggers of the Keto-Inflammatory Network

The activation of the KIN extends far beyond carbohydrate scarcity. Rather than functioning solely as a starvation-induced response, the KIN is triggered by a diverse array of physiological and pathological stressors, including systemic inflammation, microbial exposure, tissue injury, intense physical exertion, prolonged fasting, and parturition (**Figure 2**) [38–40]. These stimuli converge on evolutionarily conserved molecular pathways that coordinate metabolic adaptation with immune regulation.

At the molecular level, these triggers initiate a cascade involving mitochondrial reprogramming, AMP-activated protein kinase (AMPK) activation, peroxisome proliferator-activated receptor alpha (PPAR-α) signaling, and transcriptional networks mediated by NF-κB and FOXO1. These pathways recalibrate energy production, suppress uncontrolled inflammation, and initiate hepatic ketogenesis, placing BHB at the center of an integrated immunometabolic response.

In dairy cows, the periparturient period provides a quintessential model of KIN activation (**Figure 3**). During this time, abrupt endocrine shifts, intense lipolysis, microbial components' translocation (especially lipopolysaccharide, LPS), and elevated proinflammatory cytokines [e.g., Interleukin (IL)-1β, Tumor Necrosis Factor (TNF)-α] converge to stimulate hepatic ketogenesis alongside immune activation [5,41,42]. In humans, similar triggers, such as trauma, infection, caloric restriction, or fasting, activate hepatic oxidative metabolism through PPAR-α and sirtuin pathways, leading to increased BHB synthesis [7,40].

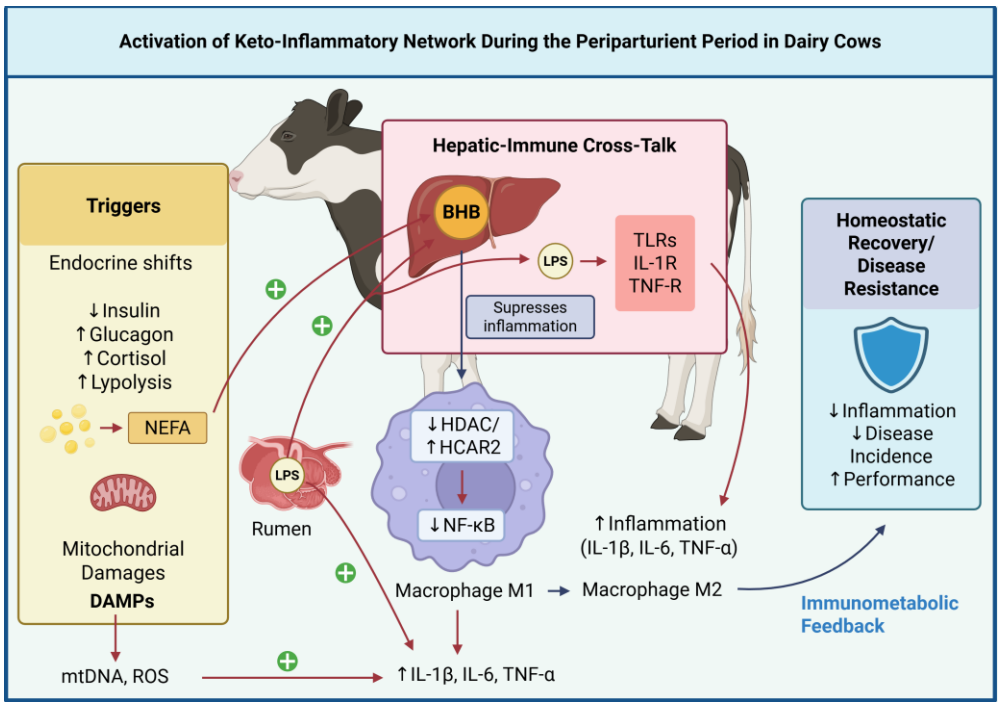

Figure 3. Activation of the Keto-Inflammatory Network During the Periparturient Period in Dairy Cows. The figure illustrates a cow undergoing physiological stress during the periparturient period. On the left, key systemic triggers are shown—endocrine shifts, lipolysis, and microbial translocation (notably LPS) , which lead to hepatic ketogenesis. The liver is depicted centrally, highlighting its role in producing β-hydroxybutyrate (BHB). This process is integrated with immune signaling through pattern recognition receptors (TLRs, IL-1R, TNF-R), which modulate inflammation. The figure shows immune cell responses, including IL-1β, IL-6, and

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TNF-α release, followed by immune suppression as BHB exerts immunoregulatory effects. Arrows demonstrate the immunometabolic feedback loop in which BHB helps temper immune activation and promote tissue resilience. The final outcome, depicted on the right, is improved physiological balance and survival through coordinated metabolic and immune adaptation. [Figure created using 2025 BioRender in collaboration with Jincheol and Jingyu Choi; (2025) https://BioRender.com/5lticxm].

Importantly, rising BHB levels often mirror elevations in IL-6 and TNF-α, suggesting a tightly coupled feedback mechanism in which ketogenesis helps suppress excessive immune activation. In this broader framework, ketogenesis is no longer a passive metabolic adaptation to nutrient depletion but a proactive and regulated mechanism of physiological resilience. It coordinates immune tone and metabolic needs in the face of diverse threats, from pathogens to parturition.

The immune system and liver engage in dynamic cross-talk during these events, linking microbial sensing (via pattern recognition receptors such as TLRs, IL-1R, and TNF-R) with hepatic substrate metabolism and mitochondrial ketone biosynthesis [43,44]. Within this integrated network, BHB emerges not as a metabolic waste product, but as a central immunometabolic signal that mediates adaptation, preserves tissue integrity, and restores homeostasis. The KIN thus represents a fundamental survival circuit, evolutionarily encoded to maintain balance during metabolic, inflammatory, and environmental perturbations.

5. Receptors and Molecular Mediators of the Keto-Inflammatory Network

The sophisticated immunomodulatory and metabolic effects of the KIN are mediated through a diverse array of molecular recognition systems and intracellular signaling pathways that enable cells and tissues to detect, interpret, and respond to ketone body availability in highly context-specific ways [6,45]. Unlike simple metabolic substrates that primarily influence cellular energetics, ketone bodies, particularly BHB, interact with multiple classes of cellular receptors, enzymes, and regulatory proteins to generate coordinated changes in gene expression, protein function, and cellular behavior (**Figure 4**) [6,7].

5.1. G-Protein Coupled Receptor Signaling

The hydroxycarboxylic acid receptor 2 (HCAR2, also known as GPR109A) represents the most extensively characterized and clinically relevant receptor system for BHB signaling [45,46]. Originally identified as a receptor for nicotinic acid, HCAR2 demonstrates high affinity binding for BHB with physiologically relevant activation occurring at concentrations typically observed during fasting, exercise, or metabolic stress [45,47]. HCAR2 expression is particularly abundant in immune cells including macrophages, neutrophils, dendritic cells, and certain T cell subsets, suggesting specialized roles in immune system modulation [45,48].

HCAR2 activation triggers Gi/Go-protein coupled signaling cascades that result in decreased intracellular cyclic adenosine monophosphate (cAMP) concentrations, leading to downstream suppression of protein kinase A activity and reduced phosphorylation of cAMP response element-binding protein (CREB) [33]. This signaling cascade ultimately results in decreased nuclear factor kappa B (NF-κB) activity, reduced transcription of pro-inflammatory cytokine genes including TNF-α, IL-1β, and IL-6, and enhanced expression of anti-inflammatory mediators such as interleukin-10 (IL-10) and transforming growth factor-β (TGF-β) [14,45,49].

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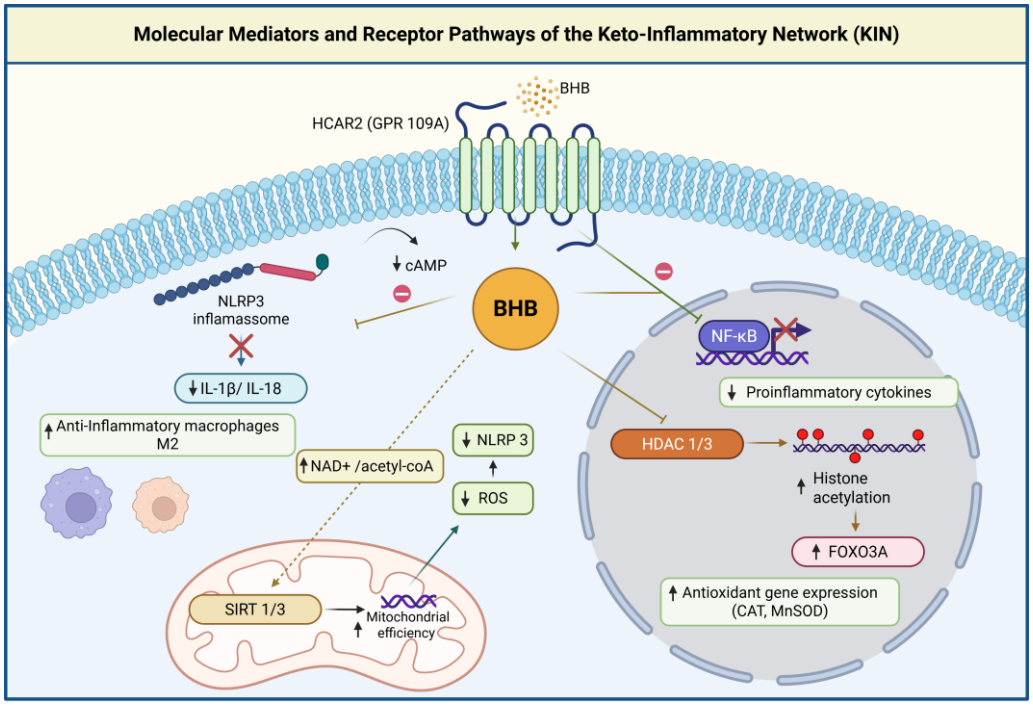

Figure 4. Molecular Mediators and Receptor Pathways of the Keto-Inflammatory Network (KIN). This diagram illustrates the key intracellular mechanisms through which β-hydroxybutyrate (BHB) orchestrates immunometabolic adaptations in the Keto-Inflammatory Network. BHB binds to the cell-surface receptor HCAR2 (GPR109A), suppressing NF-κB activation and thereby reducing transcription of pro-inflammatory cytokines. In parallel, BHB inhibits class I histone deacetylases (HDAC1 and HDAC3), enhancing histone acetylation and transcription of antioxidant and stress-response genes such as FOXO3A, catalase (CAT), and MnSOD. BHB also elevates the cellular NAD^+ pool and activates sirtuin 1 and 3 (SIRT1/3), promoting mitochondrial efficiency and redox balance. These converging pathways suppress NLRP3 inflammasome assembly, reducing IL-1β and IL-18 maturation, while favoring M2 macrophage polarization. Collectively, these responses define the molecular foundation of the Keto-Inflammatory Network, linking metabolic stress to inflammation resolution, antioxidant defense, and immune tolerance. [Created in BioRender by Jincheol and Jingyu Choi; (2025) https://BioRender.com/pyd9l4p].

5.2. Epigenetic Regulation Through Histone Deacetylase Inhibition

Among the most significant discoveries in ketone body research has been the identification of BHB as an endogenous inhibitor of class I histone deacetylases (HDACs), particularly HDAC1, HDAC2, and HDAC3 [35,50]. This mechanism of action places ketone bodies within the broader category of epigenetic modulators capable of inducing lasting changes in gene expression patterns through alterations in chromatin structure and accessibility [36,51].

The selective inhibition of class I HDACs by BHB occurs through competitive binding at the enzyme active site, with inhibition constants in the millimolar range that are readily achieved during physiological ketosis [35]. HDAC inhibition by BHB leads to increased acetylation of histones H3 and H4 at specific gene loci, resulting in chromatin relaxation and enhanced transcriptional accessibility [36,50]. Key target genes include those encoding antioxidant defense enzymes (catalase, superoxide dismutase, glutathione peroxidase), stress response transcription factors (FOXO3a, Nrf2), and anti-inflammatory mediators (IL-10, arginase 1) [52,53].

5.3. NAD^+-Dependent Sirtuin Activation

The sirtuin family of NAD^+-dependent protein deacetylases represent another critical molecular target through which ketone bodies exert regulatory effects on cellular function [54,55]. Sirtuins, particularly SIRT1 and SIRT3, play essential roles in metabolic regulation, stress resistance, and

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longevity pathways through their ability to deacetylate key regulatory proteins in both nuclear and mitochondrial compartments [56,57].

Ketone body metabolism influences sirtuin activity through multiple mechanisms including direct provision of NAD^+ cofactor, enhancement of the NAD^+/NADH ratio, and indirect effects on NAD^+ biosynthetic pathways [54,58]. SIRT1 activation by enhanced NAD^+ availability leads to deacetylation of transcription factors including p53, FOXO family members, and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), resulting in enhanced stress resistance, improved mitochondrial biogenesis, and increased oxidative metabolism [59,60].

5.4. Inflammasome Modulation and Innate Immune Signaling

The NLRP3 inflammasome represents a critical convergence point where ketone body signaling intersects with innate immune activation pathways [48,61]. BHB suppresses NLRP3 inflammasome activation through multiple complementary mechanisms including stabilization of mitochondrial membrane potential, reduction in mitochondrial reactive oxygen species (ROS) production, prevention of potassium efflux, and direct interference with inflammasome complex assembly [48,61]. These effects collectively prevent caspase-1 activation and reduce the processing and release of pro-inflammatory cytokines IL-1β and IL-18, which play central roles in sterile inflammation and metabolic dysfunction [48,62].

Metabolomics investigations have revealed that cows destined to develop ketosis show elevated concentrations of inflammatory mediators including IL-6 and TNF-α weeks before clinical disease manifestation, suggesting that inflammasome modulation represents a critical early intervention point in ketosis pathophysiology [14].

6. Central Control Nodes: Hypothalamic Integration and Circadian Coordination

The sophisticated coordination of KIN responses across multiple organ systems requires centralized integration mechanisms capable of processing diverse physiological signals and generating appropriate systemic responses [63,64]. The hypothalamus, functioning as the primary neuroendocrine control center, serves as a critical integration node where metabolic status, immune signals, circadian timing, and environmental information converge to regulate KIN activation and coordinate organism-wide adaptive responses (**Figure 5**) [63,65].

6.1. Hypothalamic Ketone Sensing and Neuroendocrine Integration

The blood-brain barrier readily permits the passage of ketone bodies, enabling direct access to hypothalamic neurons that express specialized metabolic sensing mechanisms [63,66]. Multiple hypothalamic nuclei, including the arcuate nucleus, paraventricular nucleus, and lateral hypothalamus, contain neurons that respond to changes in ketone body concentrations through alterations in membrane potential, neurotransmitter release, and neuropeptide production [63,67].

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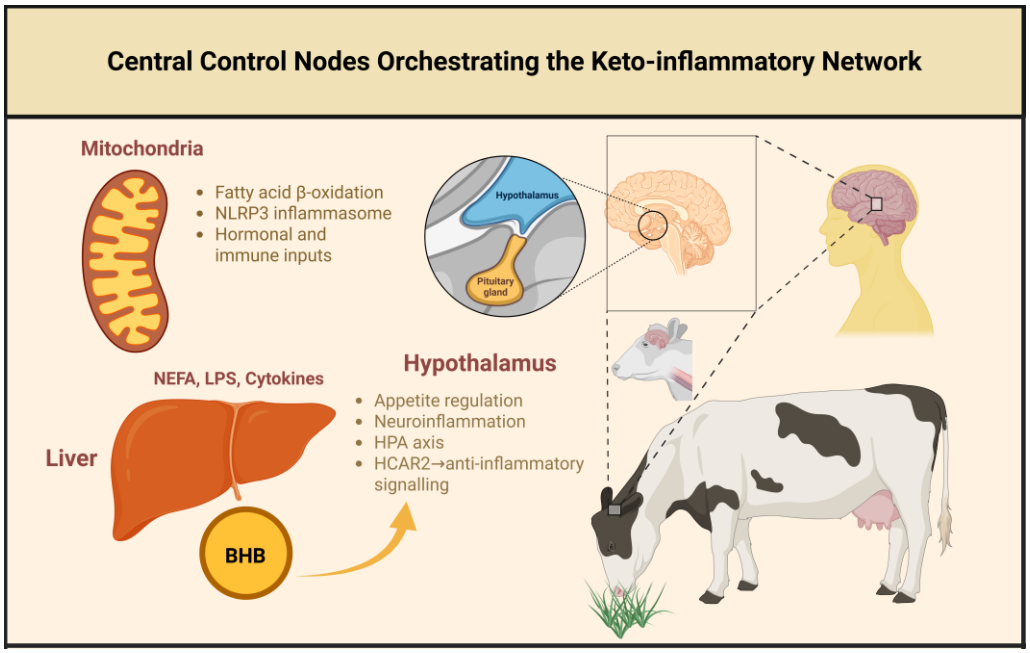

Figure 5. Central Control Nodes Orchestrating the Keto-Inflammatory Network in Cow and Human Physiology. This illustration depicts the three primary integration centers of the Keto-Inflammatory Network: the **mitochondrion**, which performs fatty acid β-oxidation, **initiates ketone synthesis**, and integrates reactive oxygen species and inflammatory signals through NLRP3 inflammasome activation; the **liver**, which processes NEFAs, LPS, and proinflammatory cytokines (e.g., TNF-α, IL-1β, IL-6) to generate **β-hydroxybutyrate (BHB);** and the **hypothalamus**, which senses peripheral BHB via **HCAR2-mediated and epigenetic signaling** to regulate appetite, neuroinflammation, and hypothalamic–pituitary–adrenal (HPA) axis activity. These control nodes are shown in both dairy cow and human, emphasizing the evolutionary conservation of immunometabolic adaptation to infection, parturition, and systemic stress. [Created in BioRender by Zohaib Saleem; (2025) https://BioRender.com/64f0lrf].

Ketone-sensitive neurons within the arcuate nucleus modulate the activity of key metabolic regulatory circuits including neuropeptide Y (NPY)/agouti-related peptide (AgRP) neurons that stimulate feeding behavior and pro-opiomelanocortin (POMC) neurons that promote satiety and energy expenditure [63]. During periods of elevated ketosis, the balance between these neuronal populations shifts to favor energy conservation through reduced thermogenesis, decreased locomotor activity, and altered feeding patterns [63,68].

6.2. Circadian Clock Integration and Temporal Coordination

The intersection of ketone metabolism with circadian biology represents a fundamental aspect of KIN function that has received increasing scientific attention [69,70]. Circadian clocks, present in virtually all cells and tissues, generate approximately 24-hour rhythms in gene expression, protein synthesis, and metabolic activity that optimize physiological function according to predictable daily cycles of feeding, fasting, activity, and rest [69,71].

Hepatic ketogenesis exhibits robust circadian rhythmicity, with peak ketone production typically occurring during the fasting phase of the daily cycle [70,72]. This temporal pattern is regulated by the coordinated expression of core clock genes including CLOCK, BMAL1, Period (PER), and Cryptochrome (CRY) that directly control the transcription of key ketogenic enzymes including HMGCS2 and enzymes involved in fatty acid oxidation [69,70]. The relationship between ketosis and circadian function extends beyond simple temporal regulation to include direct molecular interactions where ketone bodies can influence clock gene expression and circadian rhythm amplitude [58,59].

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7. Cellular Effectors of the Keto-Inflammatory Network

The functional implementation of KIN signaling occurs through coordinated responses across diverse cellular populations, each contributing specialized capabilities that collectively generate the complex physiological adaptations characteristic of effective ketotic states [73,74]. The cellular effectors of the KIN encompass both immune and non-immune cell types, with their responses being shaped by cell-specific expression patterns of ketone-sensing receptors, metabolic enzymes, and downstream signaling components (**Figure 6**) [14,73].

7.1. Myeloid Cell Populations and Innate Immunity

Macrophages represent primary cellular mediators through which the KIN exerts its anti-inflammatory and tissue-protective effects [73,75]. The functional plasticity of macrophages, encompassing a spectrum from classically activated (M1) pro-inflammatory states to alternatively activated (M2) anti-inflammatory and tissue repair phenotypes, provides an ideal cellular system for ketone-mediated immune modulation [73,74].

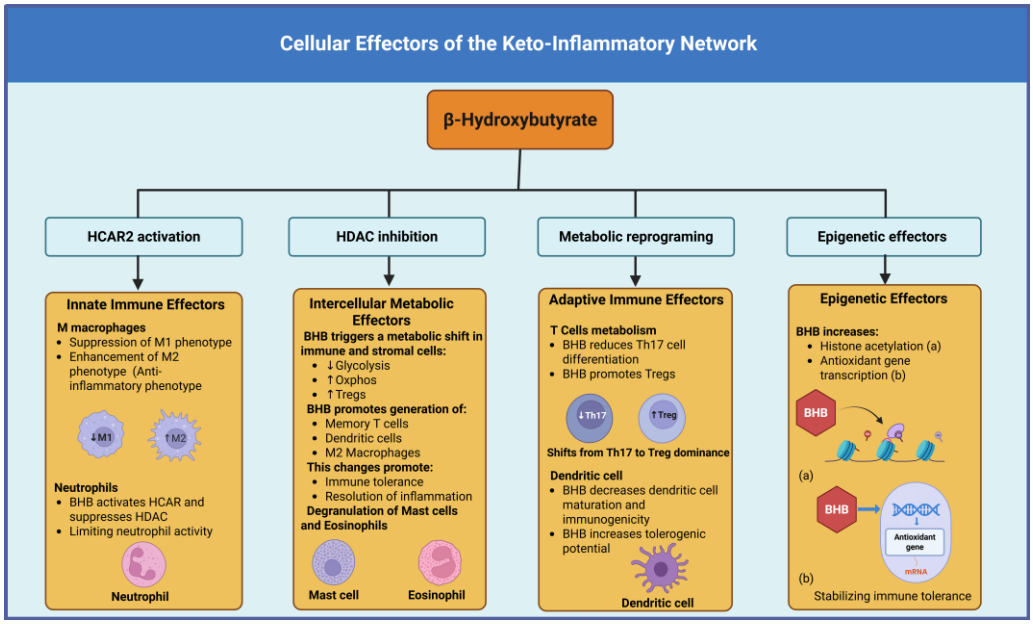

Figure 6. Cellular Effectors of the Keto-Inflammatory Network (KIN). β-Hydroxybutyrate (BHB) functions as the central signaling molecule of the KIN, acting through four principal mechanisms: HCAR2 activation, HDAC inhibition, metabolic reprograming, and epigenetic modulation. These pathways collectively regulate both innate and adaptive immune effectors. In innate cells (macrophages, neutrophils), BHB suppresses proinflammatory M1 activity, enhances M2 polarization, and limits neutrophil activation. In adaptive immunity, BHB reduces Th17 cell differentiation and promotes regulatory T cell (Treg) expansion. Metabolic reprograming suppresses glycolysis and oxidative phosphorylation (i.e., oxphos) while promoting fatty acid oxidation, supporting anti-inflammatory phenotypes in macrophages and T cells. Epigenetically, BHB enhances histone acetylation and antioxidant gene expression, stabilizing immune tolerance. Additional effector cells, including dendritic cells, mast cells, and eosinophils, contribute to immune resolution and suppression of allergic inflammation. Collectively, these cellular programs translate ketone body signaling into durable immunoregulatory states that maintain homeostasis and prevent immunopathology. [Created in BioRender by Zohaib Saleem; (2025) https://BioRender.com/tn8a1zi].

Ketone body exposure shifts macrophage polarization toward M2-like phenotypes characterized by enhanced oxidative metabolism, increased production of anti-inflammatory cytokines including IL-10 and TGF-β, elevated expression of tissue repair mediators such as arginase

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1 and insulin-like growth factor 1, and improved phagocytic clearance of apoptotic cells and cellular debris [45,76]. These changes occur through multiple molecular mechanisms including HCAR2 receptor activation, metabolic reprogramming toward oxidative phosphorylation, and epigenetic modifications that favor anti-inflammatory gene expression programs [45,77].

Remarkably, dairy cows destined to develop ketosis show elevated concentrations of inflammatory mediators including IL-6, TNF-α, and serum amyloid A beginning at -8 and -4 weeks prior to parturition, indicating that immune system activation precedes rather than follows metabolic dysfunction [14]. This temporal sequence suggests that immune system modulation represents a primary rather than secondary component of ketosis pathophysiology.

7.1. Lymphocyte Populations and Adaptive Immunity

T lymphocyte responses represent critical components of KIN function, with ketone body availability during T cell activation influencing both immediate functional responses and long-term memory formation [78,79]. The metabolic requirements of T cell activation, including the need for rapid ATP production, biosynthetic precursor generation, and sustained energy supply for proliferation and effector function, make T cells particularly sensitive to changes in substrate availability and metabolic environment [78,80].

$CD4^+$ T helper cell differentiation represents a key target for ketone-mediated immune modulation, with particular effects on the balance between pro-inflammatory Th17 cells and immunosuppressive regulatory T cells (Tregs) [81,82]. Ketone availability during T cell activation favors Treg differentiation through mechanisms including enhanced oxidative metabolism, increased expression of the transcription factor FoxP3, and epigenetic modifications that stabilize the regulatory T cell phenotype [82,83].

$CD8^+$ T cell memory formation represents another important aspect of KIN function with potential implications for vaccine responses, cancer immunotherapy, and long-term immune protection [79,84]. Memory $CD8^+$ T cells rely heavily on oxidative metabolism and fatty acid oxidation for their survival and rapid recall responses, making them well-suited to benefit from ketone body availability [79,80]. T cells activated in the presence of elevated ketone concentrations exhibit enhanced memory characteristics including improved survival, increased expression of memory-associated transcription factors, and enhanced functional capacity upon secondary antigen encounter [84,85].

8. Evolutionary Origins and Conservation of the Keto-Inflammatory Network

The remarkable conservation of ketogenic pathways and associated regulatory mechanisms across vertebrate evolution provides compelling evidence for the fundamental adaptive significance of the KIN [1,2]. Phylogenetic analyses reveal that core components of ketone metabolism, including the key biosynthetic enzymes, transport proteins, and sensing mechanisms, have been maintained with high fidelity across hundreds of millions of years of evolutionary divergence (**Figure 7**) [27,37].

8.1. Comparative Physiology Across Taxa

The utilization of ketosis as an adaptive strategy extends across diverse mammalian lineages, each demonstrating species-specific modifications that optimize ketogenic responses for particular ecological niches and life history strategies [1,86]. Arctic mammals, including seals, bears, and arctic ground squirrels, rely heavily on ketogenic metabolism during extended periods of food unavailability, with some species maintaining ketotic states for months during hibernation or migration [1,87].

Migratory species demonstrate particularly sophisticated ketogenic adaptations that support sustained locomotor performance under conditions of limited food access [8,88]. Long-distance migratory birds can maintain ketotic states throughout transcontinental flights lasting days to weeks, with ketone bodies providing a metabolically efficient fuel source that maximizes flight range while

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minimizing body weight [8,89]. The anti-inflammatory effects of ketosis during these extended periods of physical stress help prevent exercise-induced tissue damage and maintain immune function despite the physiological challenges of long-distance migration [89,90].

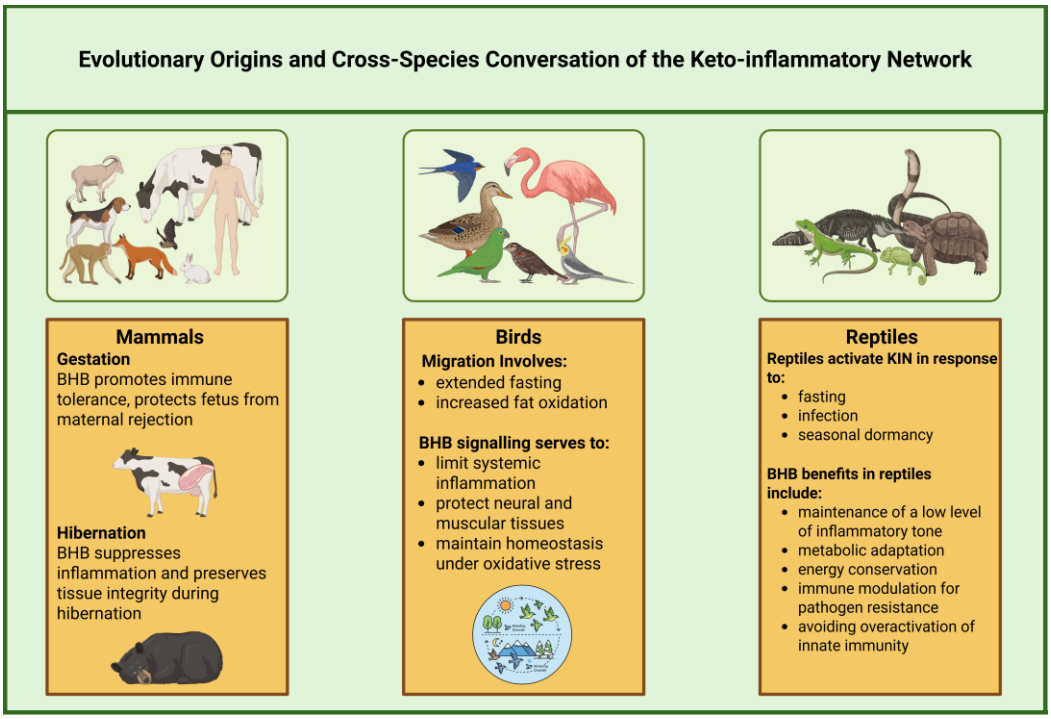

Figure 7. Evolutionary Origins and Cross-Species Conservation of the Keto-Inflammatory Network (KIN). This figure illustrates the conserved role of β-hydroxybutyrate (BHB) as a central signaling molecule within the Keto-Inflammatory Network across vertebrate taxa. In mammals, birds, and reptiles, BHB is produced during physiological stressors such as gestation, hibernation, migration, infection, and fasting. It engages conserved molecular targets - HCAR2, HDACs, sirtuins, and the NLRP3 inflammasome - to promote immune tolerance, metabolic adaptation, and anti-inflammatory homeostasis. Despite species-specific physiological strategies, the KIN functions as a universal immunometabolic system coordinating energy utilization and immune regulation in response to ecological and life-history pressures. [Created in BioRender by Zohaib Saleem; (2025) https://BioRender.com/16qs3oe].

8.2. Domestication and Artificial Selection Pressures

The intensive artificial selection applied to domestic animals, particularly dairy cattle, has created physiological challenges that may exceed the adaptive capacity of natural ketogenic responses [12,91]. Modern dairy cows produce 8-10 times more milk than their wild ancestors, creating energetic demands that can overwhelm the regulatory mechanisms that normally coordinate ketosis with immune function and metabolic adaptation [5,12]. Understanding the evolutionary context of ketogenic adaptation provides important insights for developing management strategies that work with, rather than against, natural biological processes [92,93].

9. The Calcio-Keto-Inflammatory Network: Integrating Calcium and Ketone Signaling

The intersection between calcium homeostasis and ketone metabolism represents a sophisticated regulatory interface that has profound implications for immune function, cellular signaling, and physiological adaptation [94,95]. The **Calci-Keto-Inflammatory Network (CKIN)** describes the dynamic integration of calcium-dependent signaling pathways with ketone-mediated metabolic and

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immune responses, creating emergent regulatory properties that exceed the capabilities of either system operating independently (**Figure 8**) [92,96].

Recent paradigm-shifting research has revealed that the coordinated occurrence of hypocalcemia and hyperketonemia during the periparturient period in dairy cattle may represent adaptive biological programming rather than independent pathological processes [92]. The calci-inflammatory network (CIN) provides a conceptual framework for understanding how calcium signaling coordinates with inflammatory responses to optimize physiological adaptation during periods of intense metabolic demand [92].

9.1. Advanced Molecular Mechanisms and Clinical Integration

Calcium (Ca^{2+}) serves as a ubiquitous second messenger in nearly all eukaryotic cells, governing gene transcription, cellular excitability, mitochondrial respiration, and immune activation. In immune cells, calcium influx through store-operated calcium entry (SOCE) channels - particularly those formed by STIM1 and Orai1 proteins activates transcription factors such as NFAT, NF-κB, and CREB, which drive cytokine production, cell proliferation, and survival [97,98]. Concurrently, BHB modulates immune responses by inhibiting HDACs, suppressing NLRP3 inflammasome activation, enhancing antioxidant defense, and promoting anti-inflammatory macrophage and T cell phenotypes through engagement of receptors such as HCAR2 (**Figure 9**) [38,39,99].

The mechanistic convergence between calcium and ketone signaling is particularly evident at the mitochondrial level. Mitochondria serve as central hubs for both Ca^{2+} buffering and oxidative metabolism. BHB maintains mitochondrial membrane potential, enhances respiratory efficiency, and reduces ROS generation - factors that stabilize intracellular calcium flux and protect immune cells from oxidative and inflammatory stress [44,100]. Through these effects, BHB modulates the threshold for calcium-induced immune activation and supports bioenergetic integrity.

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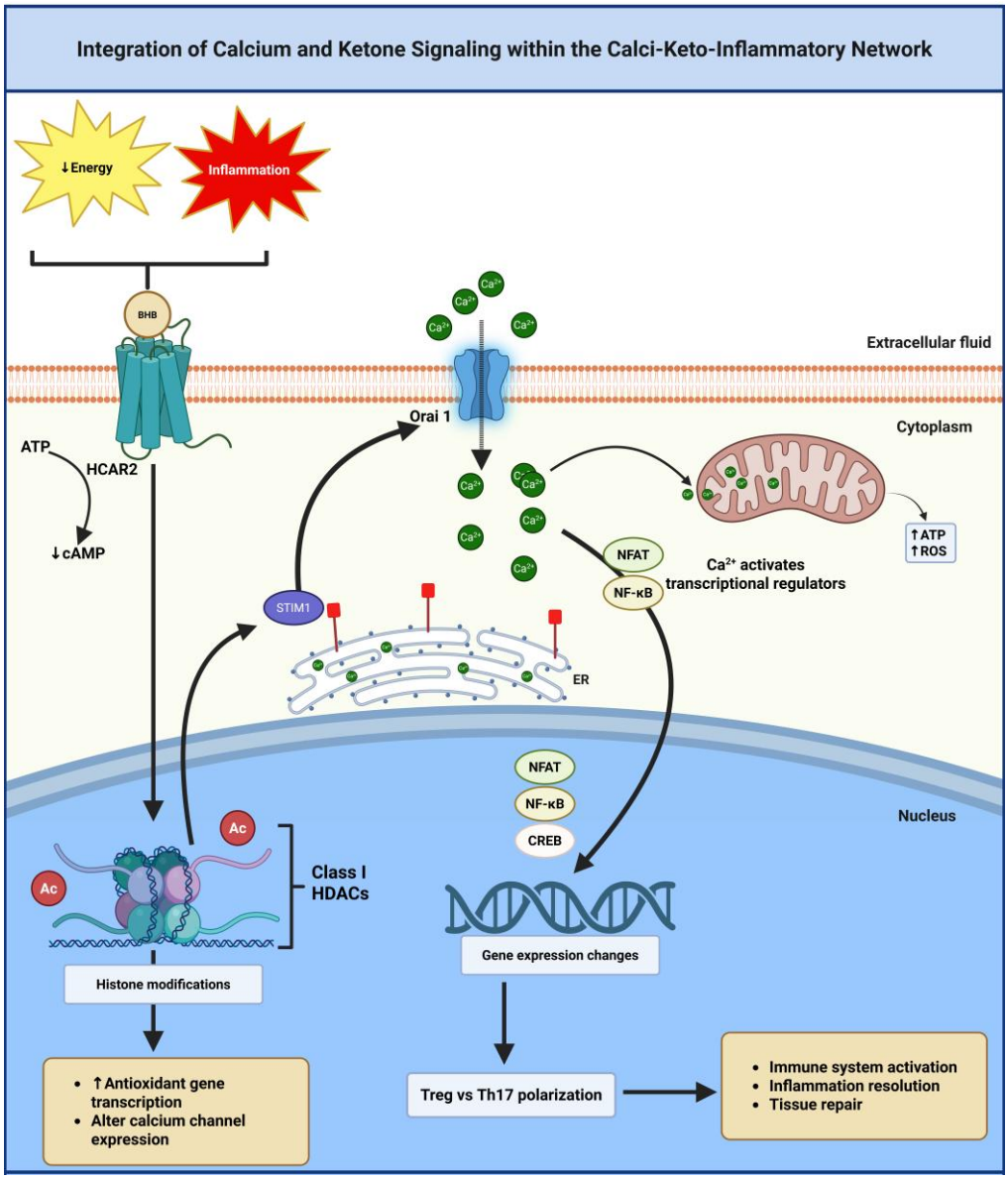

Figure 8. Integration of Calcium and Ketone Signaling within the Calci-Keto-Inflammatory Network (CKIN). This schematic illustrates the bidirectional interaction between β-hydroxybutyrate (BHB) and calcium (Ca^{2+}) signaling in the coordination of immunometabolic homeostasis. During states of energy deprivation or inflammation, BHB binds to its receptor HCAR2 (hydroxycarboxylic acid receptor 2) and inhibits class I histone deacetylases (HDAC1–3), producing epigenetic modifications that enhance antioxidant gene transcription and alter calcium channel expression. Concurrently, store-operated calcium entry (SOCE) through STIM1 and Orai1 channels activates transcription factors including NFAT, NF-κB, and CREB, which regulate T cell polarization, immune activation, inflammation resolution, and tissue repair. Mitochondrial Ca^{2+} uptake further links energy and redox balance by enhancing ATP production and ROS signaling. Together, these interconnected pathways across the ER, mitochondria, and nucleus establish the Calci-Keto-Inflammatory Network (CKIN) -a conserved system integrating energy status, calcium homeostasis, and immune regulation in both dairy cows and humans. [Created in BioRender by Zohaib Saleem; (2025) https://BioRender.com/vy5aq9s].

9.2. Clinical Applications and Therapeutic Implications

The CKIN provides a mechanistic framework for understanding the co-occurrence of hypocalcemia and hyperketonemia in periparturient dairy cows. This convergence reflects coordinated adaptive programming rather than dual pathology, with mitochondria serving as the critical integration point.

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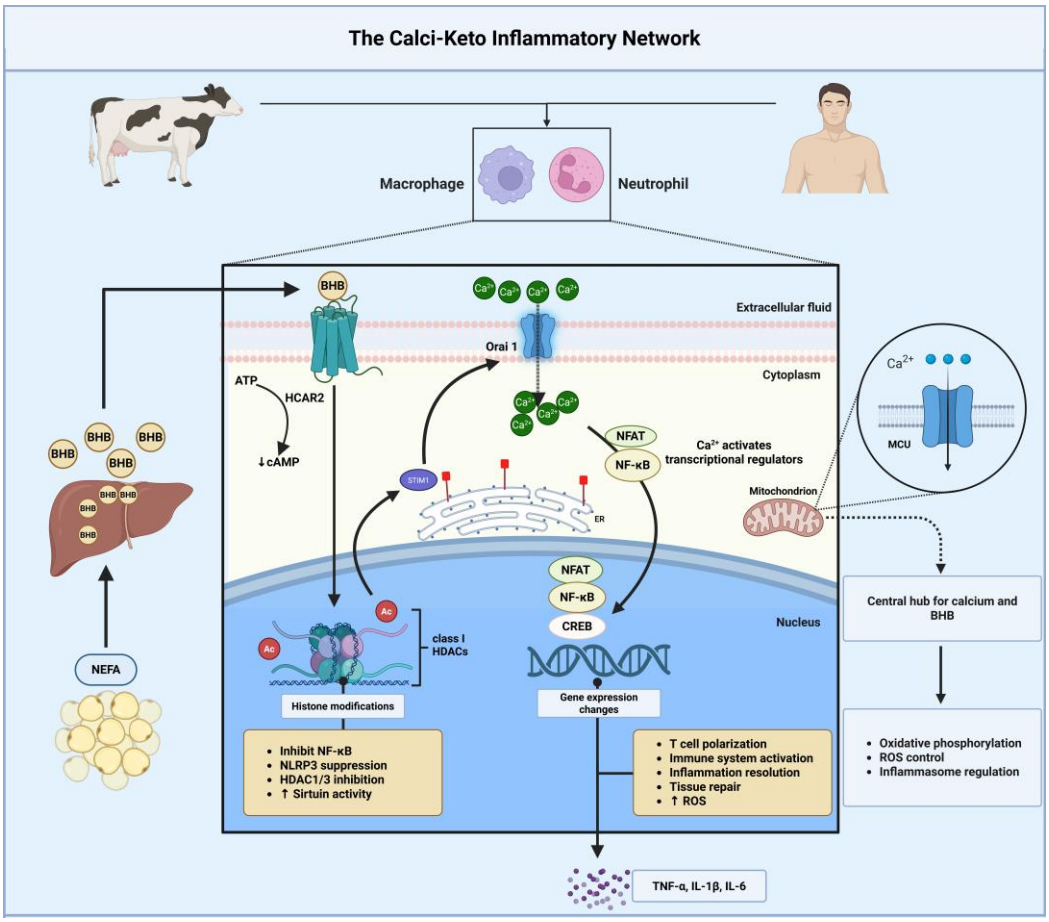

Figure 9. The Calcio-Keto-Inflammatory Network (CKIN): An Integrated Immunometabolic Framework in Dairy Cows and Humans. This diagram illustrates the coordinated cross-talk between calcium signaling and β-hydroxybutyrate (BHB) in regulating immune cell function and metabolic adaptation. In macrophages and neutrophils, store-operated calcium entry (SOCE) through STIM1 and Orai1 channels activates transcription factors NF-κB, NFAT, and CREB, driving the production of pro-inflammatory cytokines (*TNF-α*, *IL-1β*, *IL-6*). In parallel, BHB counters these responses through multiple mechanisms: binding to HCAR2 (GPR109A) to suppress NF-κB activity, inhibiting HDAC1/3 to promote anti-oxidant and anti-inflammatory gene expression, blocking NLRP3 inflammasome activation, and enhancing sirtuin activity to stabilize mitochondrial function. The mitochondria act as central hubs integrating calcium buffering and ketone metabolism to coordinate oxidative phosphorylation, ROS signaling, and inflammasome control. The liver synthesizes BHB in response to NEFA mobilization and inflammatory cues, while the hypothalamus modulates systemic immune tone and energy balance. Together, calcium and BHB function as a "pedal-and-brake" system that governs immune activation and resolution, representing an evolutionarily conserved mechanism of physiological resilience under metabolic stress. [Created in BioRender by Zohaib Saleem; (2025) https://BioRender.com/u16u95l].

Mitochondria function as central hubs integrating calcium and ketone signaling. They simultaneously buffer Ca^{2+} and conduct oxidative metabolism, with BHB playing modulatory roles across both processes. BHB maintains mitochondrial membrane potential, enhances respiratory efficiency, and reduces ROS generation - collectively stabilizing intracellular calcium flux and protecting immune cells from oxidative and inflammatory stress [44,100]. Through these mechanisms, BHB modulates the threshold for calcium-induced mitochondrial membrane permeabilization while supporting bioenergetic integration.

9.3. Clinical Applications and Therapeutic Implications

The CKIN provides a mechanistic framework for understanding the co-occurrence of hypocalcemia and hyperketonemia in periparturient dairy cows. This convergence reflects

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coordinated adaptive programming: a strategy to transiently downregulate immune reactivity, reduce metabolic strain, and allocate energy toward lactation and tissue remodeling [5,43].

In humans, disruption of this coordinated axis is increasingly recognized in the pathophysiology of chronic inflammation, metabolic syndrome, autoimmune disease, and neurodegeneration, where calcium mishandling and impaired ketogenesis co-occur [40,101]. The enhancement of CD8+ T cell memory and anti-tumor immunity through ketogenic interventions represents a particularly promising therapeutic application [97], while ketogenic approaches show remarkable efficacy in alleviating colitis through modulation of gut immune cell populations [99].

9.4. The "Pedal and Brake" System

The CKIN bridges three previously isolated domains - mineral signaling, ketone metabolism, and immune regulation - into an integrated "pedal and brake" system governing immune activation and resolution. This represents a paradigm shift: recognizing that optimal health requires understanding how energy metabolism, calcium dynamics, and immune control function as a co-evolved, adaptive network. CKIN opens new therapeutic avenues for metabolic-inflammatory diseases across species, providing a co-evolved mechanism of physiological resilience during stress [100,102].

9.5. Cellular Calcium Dynamics and Ketone Interactions

Calcium serves as a universal intracellular signaling molecule regulating diverse cellular processes including enzyme activation, gene transcription, membrane excitability, vesicle trafficking, and programmed cell death [95,97]. Ketone bodies influence cellular calcium dynamics through multiple interconnected mechanisms spanning membrane transport, intracellular buffering, and mitochondrial calcium handling [94,103].

BHB enhances the expression and activity of sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPases (SERCA pumps), leading to improved calcium sequestration within intracellular stores and enhanced cellular calcium buffering capacity [104]. Additionally, HCAR2 receptor activation by BHB modulates Ca^{2+} signaling: it activates phospholipase C and inositol 1,4,5-trisphosphate (IP3) generation, resulting in controlled calcium release from endoplasmic reticulum stores [45,105]. These dual mechanisms - enhanced sequestration and modulated release - enable BHB to stabilize calcium homeostasis during metabolic stress.

10. The Ketoinflammatory Clock: Circadian Synchronization of Immunometabolic Rhythms

Temporal coordination of metabolic and immune functions represents a fundamental organizing principle in biology, with circadian rhythm disruption contributing to metabolic syndrome, autoimmune disease, and inflammatory disorders [109,110]. The intersection of ketone metabolism with circadian biology creates the **Ketoinflammatory Clock** - a regulatory system governing daily fluctuations in immune responsiveness, metabolic flexibility, and stress adaptation (**Figure 10**) [16,69,70].

10.1. Molecular Circadian Control of Ketogenesis

Hepatic ketone body production exhibits robust circadian rhythmicity coordinated with feeding-fasting cycles [70,71]. This temporal regulation emerges from core clock genes including CLOCK, BMAL1, Period (PER), and Cryptochrome (CRY), which generate approximately 24-hour oscillations through interlocking transcriptional-translational feedback loops [69,111].

The CLOCK-BMAL1 heterodimer functions as a master transcriptional activator regulating expression of key metabolic genes including those controlling ketogenesis (HMGCS2, BDH1), fatty acid oxidation (CPT1A, ACOX1), and glucose metabolism (G6PC, PCK1) [69,111]. This molecular architecture ensures that ketogenic capacity peaks during the typical fasting period, optimizing

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metabolic efficiency. Additional circadian regulation occurs through rhythmic expression of nuclear receptors including peroxisome proliferator-activated receptor α (PPARα) and REV-ERBα, which serve as molecular links between circadian timing and metabolic regulation [69,111].

10.2. Circadian Regulation of Immune Function

Immune cells possess functional circadian clocks that autonomously regulate diverse aspects of immune function including cell trafficking patterns, activation thresholds, cytokine production kinetics, and antimicrobial activity [110,113]. The coordination of immune rhythms with metabolic cycles through the Ketoinflammatory Clock provides a mechanism for optimizing immune responses according to predicted pathogen exposure patterns and energy availability [110,114]. This temporal alignment ensures that energetically expensive immune surveillance peaks during periods of anticipated pathogen encounter (such as feeding times when microbial exposure increases), while dampening inflammatory responses during fasting periods when metabolic resources are directed toward cellular maintenance and repair. The integration of ketone signaling with circadian immune regulation thus enables strategic allocation of metabolic and immune resources across the 24-hour cycle, balancing pathogen defense against metabolic efficiency.

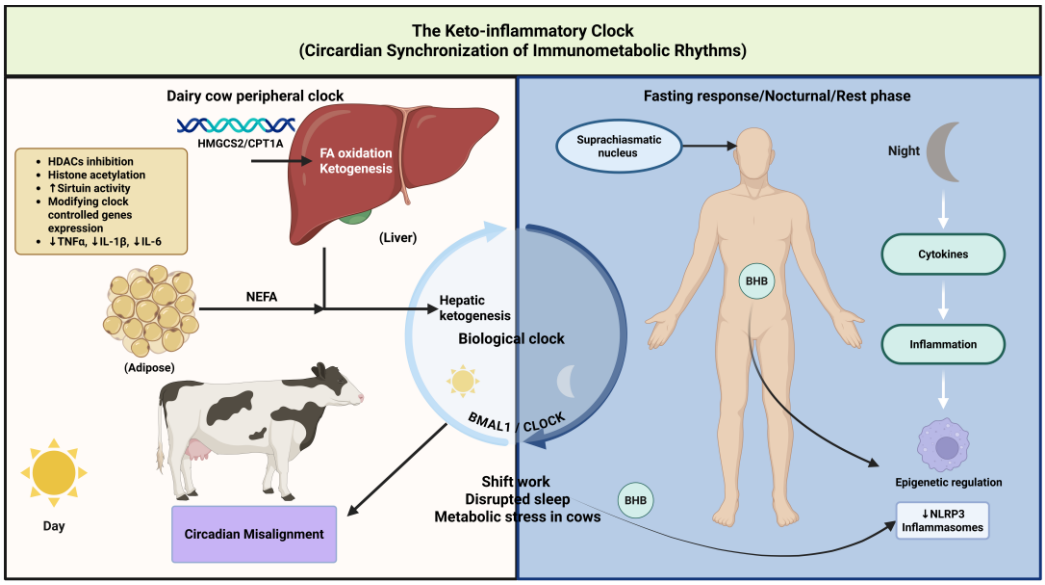

Figure 10. The Ketoinflammatory Clock: Circadian Coordination of Ketone Metabolism and Immunoinflammatory Rhythms in Humans and Dairy Cattle. This conceptual model illustrates the bidirectional interactions between the circadian clock, hepatic ketogenesis, and immunometabolic regulation, termed the Ketoinflammatory Clock. Environmental time cues (light–dark cycles, feeding patterns, and sleep rhythms) entrain both central (suprachiasmatic nucleus, SCN) and peripheral clocks in the liver, adipose tissue, and immune cells. Core circadian transcription factors, BMAL1 and CLOCK, drive the rhythmic expression of ketogenic enzymes including HMGCS2 and CPT1A, thereby promoting fatty acid oxidation and β-hydroxybutyrate (BHB) production during fasting or the nocturnal/rest phase. In turn, BHB functions as a chrono-metabolic signal: it inhibits histone deacetylases (HDACs), enhances histone acetylation, activates sirtuins, and modulates the expression of clock-controlled genes involved in immune regulation. At the cellular level, BHB suppresses NLRP3 inflammasome activation, dampens proinflammatory cytokine production (e.g., TNF-α, IL-1β, IL-6), and contributes to immune homeostasis through epigenetic reprogramming. Disruption of circadian alignment—through shift work, sleep disturbances, or periparturient metabolic stress in dairy cows—impairs ketone signaling, leading to reduced BHB levels, circadian desynchrony, and heightened inflammatory responses. This diagram highlights key metabolic tissues (liver, adipose), molecular feedback loops, and BHB-mediated immunoepigenetic effects, illustrating a systems-level mechanism that links timekeeping to metabolic flexibility and inflammatory resilience. The Ketoinflammatory Clock provides a framework for designing

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circadian-aligned therapeutic and nutritional strategies to support immune balance in both humans and ruminants. [Created in BioRender by Zohaib Saleem; (2025) https://BioRender.com/ihutbsa].

11. The Ketosis-Microbiota-Immune Triad: Trans-Kingdom Signaling

The recognition that host metabolism and microbial ecology function as integrated systems has fundamentally transformed our understanding of health, disease, and metabolic regulation [34,115]. The ketosis-microbiota-immune triad exemplifies trans-kingdom signaling, where host-derived ketone bodies and microbe-derived metabolites converge on shared regulatory pathways to coordinate systemic physiological responses (**Figure 11**) [81,116].

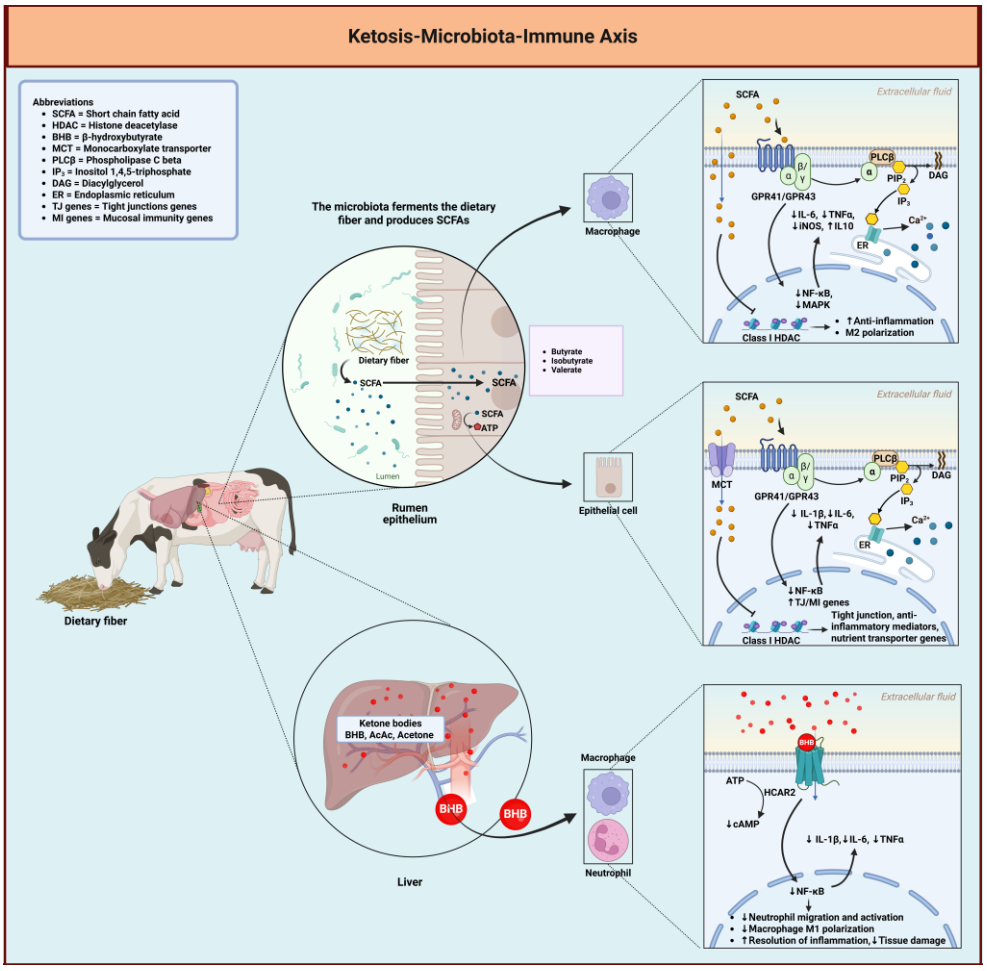

Figure 11. Ketosis–Microbiota–Immune Axis: Parallel Anti-Inflammatory Mechanisms of SCFAs and Ketone Bodies. This diagram illustrates how gut-derived short-chain fatty acids (SCFAs) and liver-derived β-hydroxybutyrate (BHB) suppress proinflammatory immune responses. Microbial fermentation of dietary fiber in the gut produces SCFAs (e.g., butyrate, isobutyrate, valerate), which are absorbed by epithelial cells via MCT transporters and activate G-protein coupled receptors (GPR41/43), triggering inhibition of histone deacetylases (HDACs) and the NF-κB signaling pathway. This leads to decreased expression of IL-1β, IL-6, and TNF-α and reinforcement of epithelial barrier function. Simultaneously, hepatic ketogenesis generates BHB, which binds to HCAR2 on macrophages and neutrophils, lowering cAMP levels and suppressing NF-κB–mediated transcription. Both SCFAs and BHB dampen immune activation and cytokine release, promoting macrophage M2 polarization, resolution of inflammation, and maintenance of tissue homeostasis. [Created in BioRender by Zohaib Saleem; (2025) https://BioRender.com/9mtizvh].

11.1. Evolutionary Origins of Host-Microbe Metabolic Integration

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The intimate metabolic relationship between mammals and their microbial communities reflects millions of years of co-evolution that has shaped both host physiology and microbial metabolic capabilities [34,116]. The structural similarity between host-derived BHB and microbe-derived butyrate exemplifies this evolutionary convergence [117,118]. Both molecules activate HCAR2 receptors despite their distinct biosynthetic origins, suggesting that host signaling systems evolved to recognize and respond to functionally similar metabolites regardless of source [45,82].

11.2. Bidirectional Metabolic Communication

Host-derived ketone bodies directly influence microbial growth, with some bacterial species demonstrating enhanced growth while others are inhibited in the presence of ketones [119,120]. Ketogenic dietary interventions consistently alter microbiome composition, including reduced abundance of pro-inflammatory taxa such as Enterobacteriaceae and increased representation of beneficial genera including Akkermansia, Bifidobacterium, and Lactobacillus [114,120].

Conversely, microbial metabolites significantly influence host ketogenic capacity. Short-chain fatty acids produced by microbial fermentation of dietary fiber enhance hepatic ketogenesis through activation of AMPK and PPARα, key regulators of fatty acid oxidation and ketone production [121,122]. The microbiota also influences ketogenesis through effects on systemic inflammation, with balanced microbial communities maintaining intestinal barrier integrity and limiting LPS translocation that can suppress hepatic ketogenic capacity [49,116].

12. Ketosis and Immuno-Epigenetic Memory

The concept of immunological memory has traditionally focused on the adaptive immune system's capacity to retain information about previous antigen encounters through long-lived memory T and B cells [123]. However, emerging evidence demonstrates that the innate immune system also possesses memory-like properties through epigenetic reprogramming mechanisms that persistently alter immune cell responsiveness [85]. Ketone body exposure represents a particularly potent stimulus for establishing beneficial epigenetic memory that enhances resistance to inflammatory challenges and improves stress adaptation (**Figure 12**) [35,36].

12.1. Molecular Mechanisms of Ketone-Induced Epigenetic Memory

The establishment of epigenetic memory by ketone bodies involves coordinated modifications to chromatin structure that persist long after ketone concentrations return to baseline levels [35,50]. The primary mechanism involves BHB-mediated inhibition of class I histone deacetylases, leading to increased acetylation of histones H3 and H4 at specific gene loci involved in stress response, antioxidant defense, and anti-inflammatory signaling [35,36].

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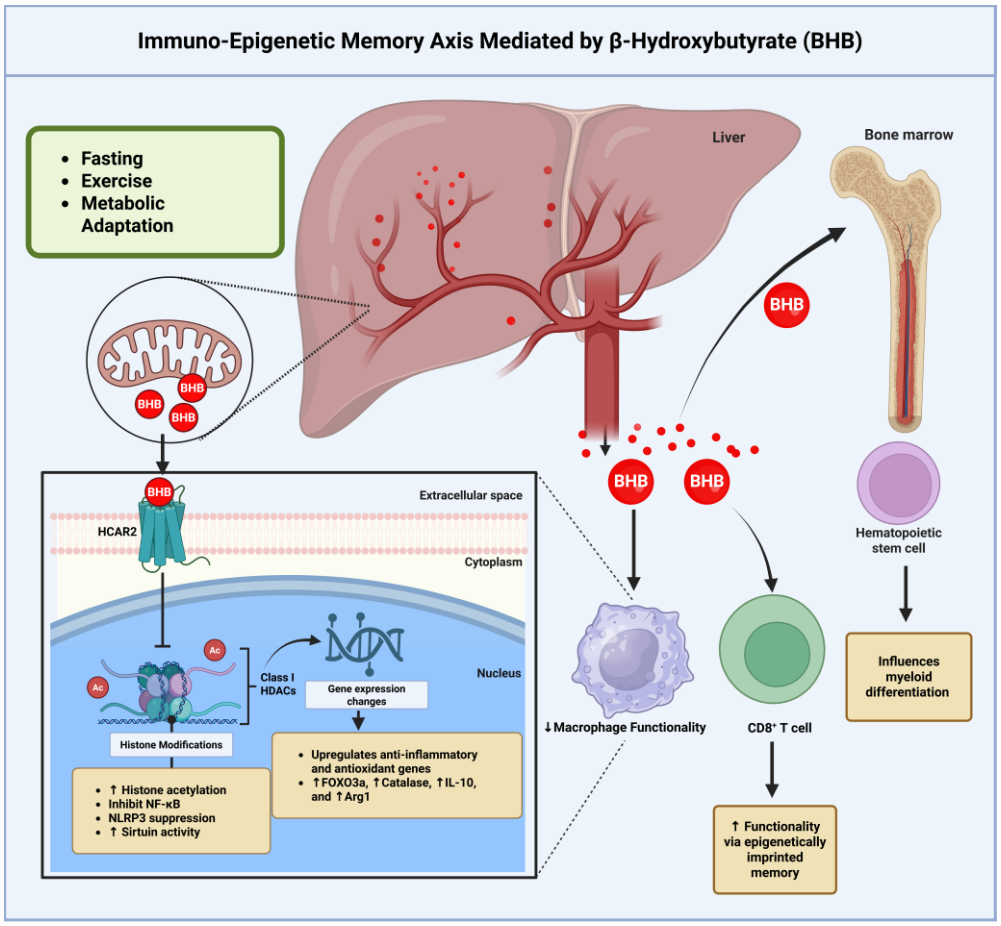

Figure 12. Immuno-Epigenetic Memory Mediated by β-Hydroxybutyrate (BHB). This figure illustrates the capacity of BHB, a key ketone body, to induce durable epigenetic changes in immune cells. During ketosis (e.g., triggered by fasting, exercise, or metabolic adaptation), mitochondria generate BHB, which enters cells and inhibits class I HDACs, leading to increased histone acetylation. This epigenetic remodeling upregulates anti-inflammatory and antioxidant genes such as FOXO3a, catalase, IL-10, and Arg1. These transcriptional effects contribute to immune tolerance and reprogramming of innate immune cells. In hematopoietic stem cells, BHB also influences myeloid differentiation and enhances the function of CD8+ T cells through epigenetically imprinted memory. This mechanistic pathway links metabolic adaptation to immunological plasticity and anti-inflammatory resilience, supporting the concept of BHB as a central metabolic mediator of long-term immuno-epigenetic memory. [Created in BioRender by Zohaib Saleem; (2025) https://BioRender.com/95wpx0g].

Key target genes for ketone-induced epigenetic modifications include those encoding antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase), stress-response transcription factors (FOXO3a, Nrf2), anti-inflammatory cytokines (IL-10), and tissue repair mediators (arginase 1, TGF-β) [52,53]. The coordinated upregulation of these protective gene programs creates cellular phenotypes characterized by enhanced resilience to oxidative stress, reduced inflammatory responsiveness, and improved capacity for tissue repair and regeneration [102,104].

12.2. Trained Immunity and Metabolic Reprogramming

Unlike classical trained immunity paradigms that typically involve enhanced inflammatory responses, ketone-induced immune training promotes metabolic reprogramming toward oxidative metabolism and anti-inflammatory programming [75,85]. Monocytes and macrophages exposed to ketone bodies demonstrate lasting metabolic changes including enhanced oxidative phosphorylation, increased fatty acid oxidation, and improved mitochondrial biogenesis [75,76]. The effects of ketone-induced trained immunity extend to hematopoietic stem and progenitor cells within the bone

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marrow, where epigenetic modifications can influence the differentiation programs that generate mature immune cells [85].

13. The Ketosis-Liver-Immune Triad: Hepatic Surveillance and Metabolic Integration

The liver occupies a unique position in mammalian physiology as the primary site of ketone body synthesis, a major organ for immune surveillance and regulation, and a critical integration hub for metabolic and inflammatory signaling [124,125]. The hepatic implementation of KIN responses involves sophisticated coordination between hepatocyte metabolic activity, resident immune cell populations, and circulating immune mediators that collectively determine both ketogenic capacity and systemic immune tone (**Figure 13**) [13,124].

13.1. Hepatic Architecture and Metabolic-Immune Integration

The structural organization of the hepatic lobule creates distinct metabolic and immunological microenvironments that contribute to the sophisticated regulation of ketogenic responses [124,126]. Hepatocytes are arranged in plates extending from portal triads to central veins, creating oxygen and nutrient gradients that influence both metabolic enzyme expression and immune cell distribution [125,126].

The liver contains the largest population of tissue-resident macrophages (Kupffer cells) in the body, strategically positioned within hepatic sinusoids to monitor portal blood for potential threats including pathogens, toxins, and inflammatory mediators [49,110]. During periods of increased ketogenesis, Kupffer cells demonstrate altered activation patterns characterized by reduced inflammatory responsiveness and enhanced tissue repair functions [13,76].

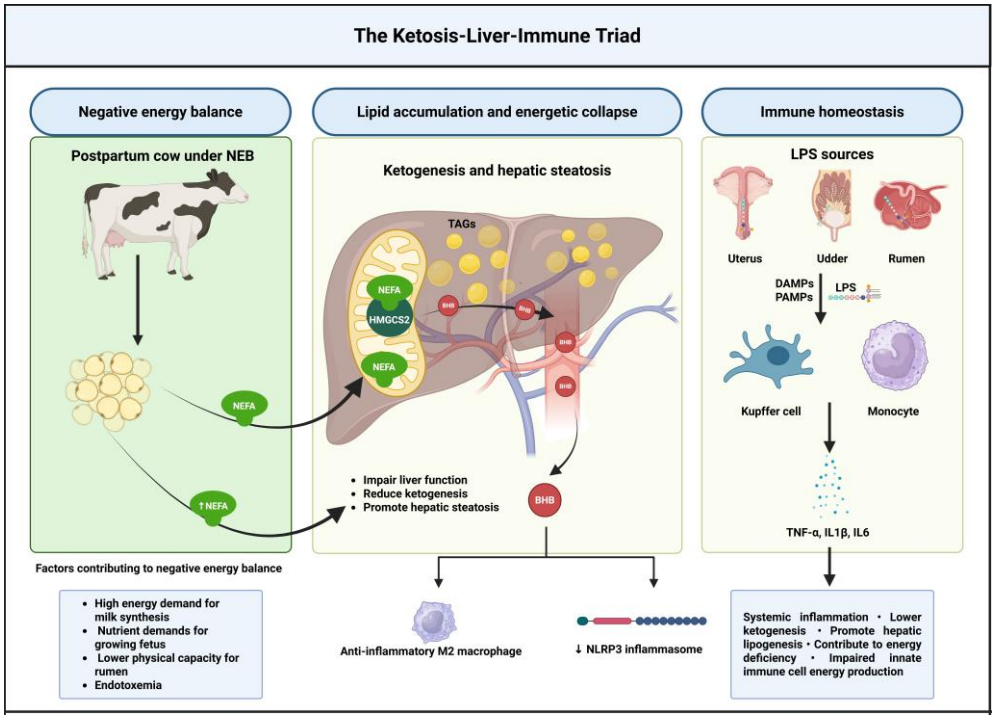

Figure 13. The Ketosis-Liver-Immune Triad. **Immunometabolic cascade linking negative energy balance (NEB), hepatic steatosis, and inflammatory dysregulation in postpartum dairy cows.** This figure illustrates the interconnected metabolic and immune disturbances occurring during early lactation in dairy cows. Under negative energy balance (NEB), driven by high milk energy demand, reduced feed intake, and systemic inflammation, adipose lipolysis releases large amounts of non-esterified fatty acids (NEFA) into circulation. Excessive NEFA uptake by the liver overwhelms β-oxidation and ketogenesis pathways regulated by CPT1A

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and HMGCS2, leading to triglyceride (TAG) accumulation, hepatic steatosis, and decreased β-hydroxybutyrate (BHB) production. Concurrently, lipopolysaccharides (LPS) derived from the rumen, uterus, and udder activate Kupffer cells and monocytes, inducing NLRP3 inflammasome activation and release of proinflammatory cytokines (TNF-α, IL-1β, IL-6). This systemic inflammation further suppresses hepatic oxidative metabolism and ketogenesis, enhancing lipogenesis and perpetuating energy deficiency. The figure depicts a self-reinforcing immunometabolic loop in which lipid mobilization, hepatic dysfunction, and LPS-driven inflammation collectively impair energy balance, liver performance, and immune homeostasis in transition dairy cows. [Created in BioRender by Zohaib Saleem; (2025) https://BioRender.com/3djx8y3].

13.2. Pathological Disruption and Therapeutic Restoration

Hepatic dysfunction can significantly compromise KIN function through impaired ketogenic capacity, altered immune regulation, or disrupted integration of metabolic and inflammatory signals [127,128]. Fatty liver disease, increasingly prevalent in periparturient dairy cattle and increasingly prevalent in humans, can impair ketogenic enzyme expression and activity while promoting hepatic inflammation [13,94].

Metabolomics investigations have revealed that alterations in liver function markers and lipid metabolism pathways are detectable weeks before clinical ketosis manifestation, suggesting that hepatic dysfunction represents an early and potentially reversible component of ketosis pathophysiology [93]. Therapeutic approaches that support hepatic KIN function focus on optimizing the balance between fatty acid delivery, oxidative capacity, and ketogenic activity [127,129].

14. The Mitochondrial Interface of Metabolic-Immune Integration

Mitochondria represent the fundamental cellular interface where energy metabolism and immune signaling converge to determine cellular fate decisions, stress responses, and functional adaptations [17,62]. Within the context of the KIN, mitochondria serve simultaneously as the primary sites of ketone body production and utilization, critical regulators of immune cell activation and function, and central integrators of metabolic and inflammatory signaling (**Figure 14**) [17,94].

14.1. Mitochondrial Ketone Metabolism and Bioenergetics

The mitochondrial matrix contains the complete enzymatic machinery for both ketone body synthesis and oxidation, enabling mitochondria to function as both producers and consumers of ketone bodies depending on cellular metabolic status and tissue-specific requirements [4,130]. The regulation of mitochondrial ketogenesis involves sophisticated control mechanisms that integrate information about substrate availability, energy demand, and cellular stress status [27,130].

In peripheral tissues, mitochondria serve as the primary sites of ketone body oxidation through the action of succinyl-CoA:3-ketoacid CoA transferase (SCOT) and acetyl-CoA acetyltransferase [102,131]. The energy yield from ketone oxidation, combined with the metabolic efficiency of ketone utilization, makes ketone bodies particularly valuable fuel sources for metabolically active cells including immune cells, cardiac myocytes, and neurons [131].

14.2. Immune Cell Metabolic Reprogramming

Different immune cell populations demonstrate distinct metabolic requirements reflecting their specialized functional roles [78,79]. Activated effector T cells rely heavily on glycolysis to support rapid proliferation and cytokine production, while memory T cells depend on oxidative phosphorylation and fatty acid oxidation for longevity and rapid recall responses [78,80].

Ketone body availability can significantly influence immune cell metabolic programming by providing alternative oxidative substrates that favor anti-inflammatory and memory cell phenotypes [75,77]. Macrophages cultured in the presence of ketone bodies demonstrate enhanced oxidative metabolism, reduced glycolytic activity, and increased expression of M2 activation markers including

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arginase 1, IL-10, and TGF-β [73,76]. This metabolic shift is consistent with the observation that cows destined to develop ketosis show early alterations in immune cell function and inflammatory mediator production [14].

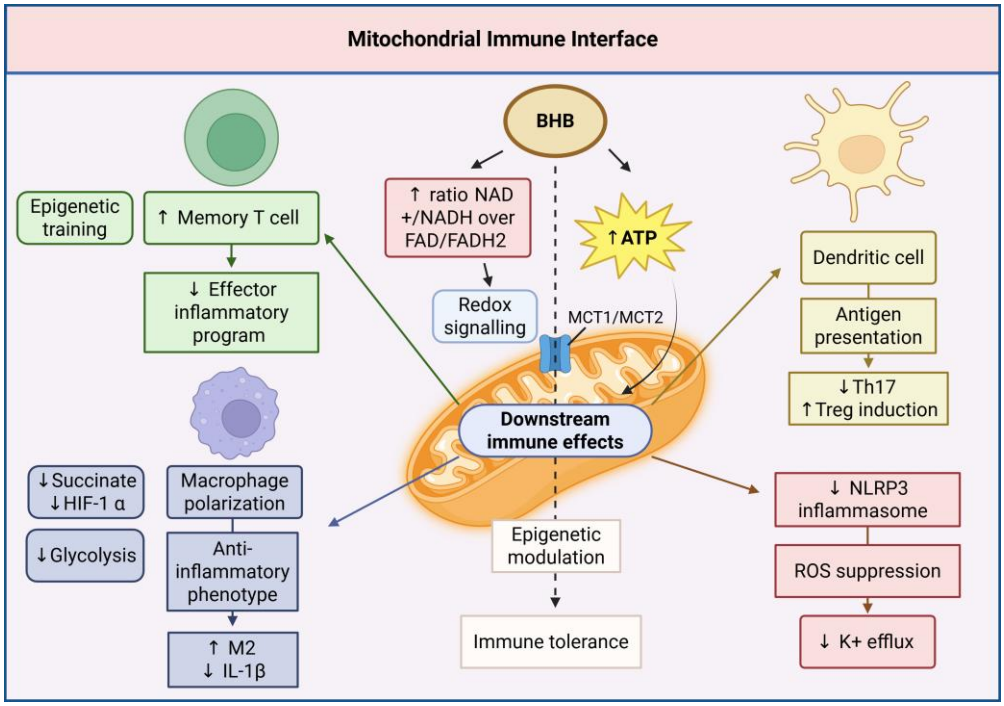

Figure 14. *Mitochondrial Immune Interface.* This diagram illustrates the central role of mitochondria as immunometabolic integrators during ketosis. β-hydroxybutyrate (BHB), the predominant ketone body, enters immune cells via monocarboxylate transporters and fuels mitochondrial oxidative phosphorylation (OXPHOS), increasing ATP synthesis and altering redox balance through enhanced NAD^+/NADH and FAD/$FADH_2$ ratios. These redox and energetic shifts reprogram immune cell metabolism, suppressing glycolysis and stabilizing anti-inflammatory phenotypes. In macrophages, this favors M2-like polarization, reduces IL-1β production, and prevents NLRP3 inflammasome activation by limiting mitochondrial ROS and potassium efflux. In T cells, the mitochondrial interface promotes memory T cell formation while dampening effector inflammatory programs. In dendritic cells, elevated mitochondrial respiration supports antigen presentation and skews T cell differentiation toward regulatory T cells (Tregs) while limiting Th17 polarization. These mitochondrial changes also influence epigenetic remodeling, contributing to immune tolerance and long-term anti-inflammatory programming. Together, this mitochondrial interface defines a critical node for integrating metabolic and immune signals during ketotic states. [Created in BioRender by Jincheol and Jingyu Choi; (2025) https://BioRender.com/4evbqh3].

15. The Ketosis-Placenta-Offspring Triad: Developmental Programming and Transgenerational Effects

The maternal-fetal interface represents a critical developmental window where maternal metabolic status profoundly influences fetal growth, organ development, and long-term health outcomes [5,132]. Maternal ketosis during pregnancy creates unique opportunities for developmental programming through direct effects of transplacental ketone transfer, epigenetic modifications in developing tissues, and alterations in maternal immune function that influence fetal immune development (**Figure 15**) [133].

15.1. Placental Ketone Transport and Metabolism

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The placenta functions as a sophisticated metabolic organ that actively regulates the transfer of nutrients, metabolites, and signaling molecules between maternal and fetal circulation [132]. Ketone bodies cross the placental barrier through specialized monocarboxylate transporters (MCTs), particularly MCT1 and MCT4, which are expressed on both maternal and fetal sides of the placental interface [102].

15.2. Fetal Ketone Utilization and Neurodevelopment

During fetal development, ketone bodies serve multiple functions that extend beyond simple energy provision to include roles in brain development, myelination, and neurotransmitter synthesis [134,135]. The developing fetal brain demonstrates particularly high capacity for ketone utilization, with ketone oxidation providing up to 25-30% of total brain energy requirements during certain developmental stages [131,134].

15.3. Epigenetic Programming and Immune Development

Maternal ketosis can influence fetal development through epigenetic mechanisms that establish lasting changes in gene expression patterns [36,133]. The developing epigenome is particularly susceptible to environmental influences during critical developmental windows, and ketone-mediated HDAC inhibition can establish epigenetic modifications that persist throughout life [35,133]. The immune system represents one of the most important targets for developmental programming by maternal ketones [83].

16. Reframing Ketosis: From Pathology to Adaptive Programming

The fundamental reconceptualization of ketosis from a pathological condition requiring correction to an adaptive biological program deserving support represents one of the most significant paradigm shifts in modern metabolic medicine and veterinary science [5,6,11]. This transformation in understanding requires moving beyond reductionist approaches that classify any deviation from baseline metabolism as inherently problematic, instead embracing the sophisticated biological complexity that characterizes ketotic adaptation [124,135].

16.1. Historical Context and Conceptual Evolution

The medical and veterinary establishment's historical perspective on ketosis has been profoundly shaped by observations made primarily within extreme pathological contexts where massive ketone elevations were associated with life-threatening conditions including diabetic ketoacidosis and starvation ketosis in humans as well as severe postpartum clinical ketosis in dairy cattle [10,18]. This pathocentric perspective was reinforced by the limited analytical capabilities available during the early development of clinical biochemistry [10,22].

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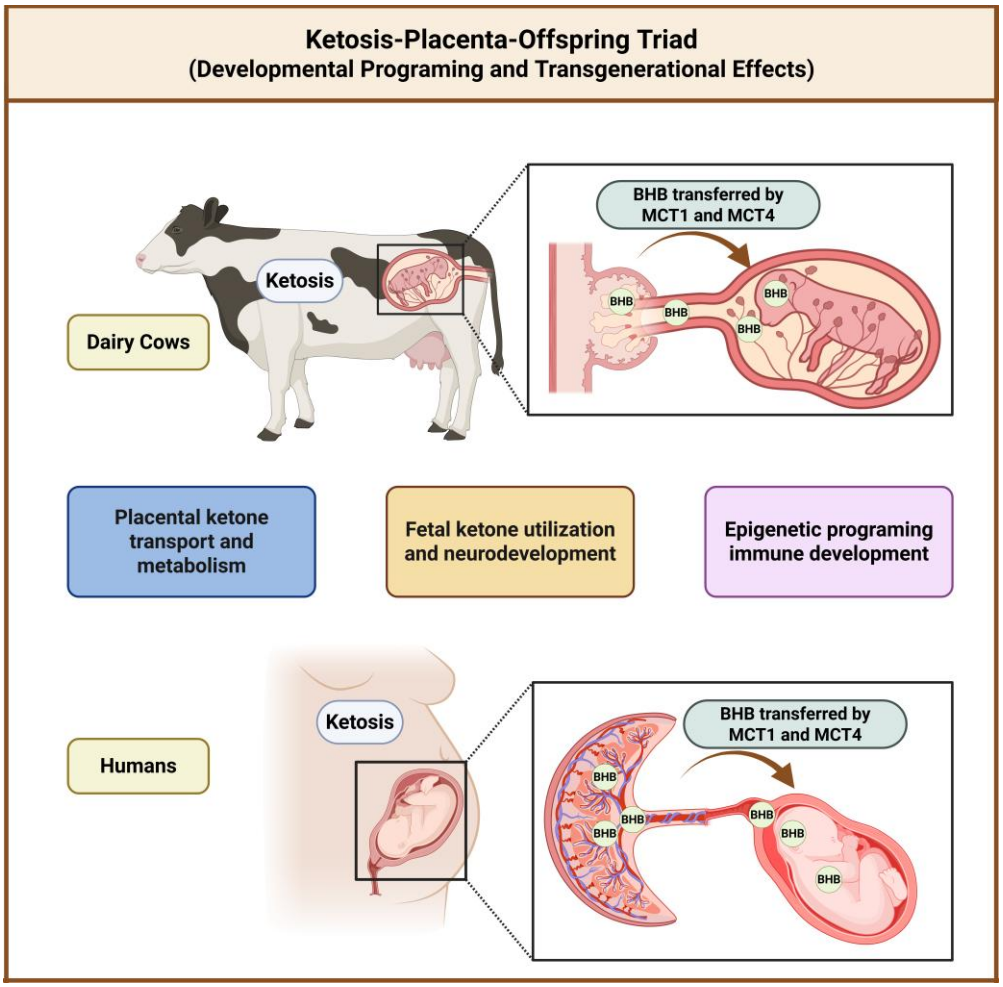

Figure 15. *Ketosis-Placenta-Offspring Triad: Developmental Programing and Transgenerational Effects in Humans and Dairy Cattle.* Maternal ketosis - whether physiological (e.g., fasting, late gestation) or pathological (e.g., periparturient ketosis, diabetes) - elevates circulating β-hydroxybutyrate (BHB), which readily crosses the placenta through monocarboxylate transporters (MCT1 and MCT4). Within the fetal compartment, ketones serve as essential energy substrates for brain development, myelination, and neurotransmitter biosynthesis. Beyond their metabolic role, ketones function as epigenetic effectors by inhibiting class I histone deacetylases (HDAC 1–3), thereby increasing histone acetylation and modulating transcription of genes governing immune maturation and stress resilience. These mechanisms underpin developmental programming, wherein maternal metabolic states influence offspring immune function and disease susceptibility. In ruminants, including dairy cows, comparable placental transport and epigenetic processes likely influence calf growth, immune competence, and long-term productivity, reflecting conserved principles of transgenerational metabolic adaptation. [Created in BioRender by Zohaib Saleem; (2025) https://BioRender.com/hzbfi5a].

The evolution toward a more balanced understanding of ketosis has been driven by technological advances that enable precise measurement of ketone concentrations, sophisticated analytical techniques that can characterize the molecular mechanisms of ketone action, and longitudinal studies that reveal the complex relationships between ketosis and health outcomes [5,6,93]. Metabolomics investigations have been particularly valuable in revealing the complex metabolic alterations that precede, accompany, and follow ketosis development, demonstrating that ketosis represents coordinated physiological programming rather than simple metabolic failure [93].

16.2. Physiological vs. Pathological Ketosis: Critical Distinctions

The recognition of ketosis as an adaptive response does not negate the reality that ketotic states can become pathological under certain circumstances [11,19]. The critical distinction lies not in the simple presence or absence of ketone bodies, but rather in the physiological context, magnitude,

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duration, and associated metabolic conditions that determine whether ketosis serves beneficial or harmful functions [135,136].

Predictive metabolomics approaches have demonstrated that it is possible to identify cows at risk for developing pathological ketosis weeks before clinical manifestation, enabling targeted interventions that could support beneficial adaptive ketotic responses while preventing pathological progression [136]. These findings suggest that the traditional reactive approach to ketosis management could be replaced with proactive strategies that optimize natural adaptive mechanisms.

16.3. Therapeutic Implications of the Adaptive Paradigm

The recognition that ketosis can serve adaptive functions has profound implications for therapeutic approaches across multiple medical and veterinary disciplines [11]. Rather than focusing exclusively on ketone suppression, optimal therapeutic strategies should aim to support the beneficial aspects of ketotic adaptation while addressing the factors that prevent healthy ketogenic regulation [30,127].

This paradigm shift is consistent with the growing recognition that many metabolic "diseases" may represent adaptive responses to environmental challenges that become pathological only when the adaptive capacity is exceeded or when the adaptive response persists beyond its optimal duration [5]. The challenge for clinicians and researchers is to develop approaches that can distinguish between beneficial and harmful aspects of metabolic adaptation and to design interventions that support optimal physiological function.

17. The Calci-Keto-Inflammatory Code: A Systems Integration Framework

The synthesis of evidence presented throughout this review converges on a fundamental reconceptualization: ketosis represents not merely a metabolic disorder caused by energy deficiency, but rather an integral component of a sophisticated biological information processing system. We propose the **Calci-Keto-Inflammatory Code** (**CKIC**) as a unifying framework that integrates calcium homeostasis, ketone metabolism, and immune regulation into a coordinated system capable of encoding, transmitting, and decoding physiological information.

The transition from viewing these processes as independent "pathways" to recognizing them as an integrated "code" is conceptually significant. While network terminology properly describes interconnections between metabolic and inflammatory systems, it inadequately captures the fundamental information-theoretic properties we now recognize as central to biological regulation. A code, by definition, requires vocabulary (molecular signals), grammar (temporal dynamics), semantics (biological meaning), and pragmatics (context-dependent interpretation) [32,33]. Preliminary evidence suggests that mammalian metabolic-immune systems exhibit precisely these properties, with calcium functioning as a master regulator that gates cross-talk between subsystems [137] [manuscript in preparation].

17.1. Calcium as a Master Regulatory Node

Central to this framework is recognition of calcium's dual function as both metabolic signal and immune regulator. Beyond its well-established roles in muscle contraction, nerve transmission, and bone metabolism, calcium serves a previously underappreciated function as a dynamic immune rheostat [92]. Elevated extracellular calcium concentrations directly activate macrophages and neutrophils through calcium-dependent signaling pathways, stimulating pro-inflammatory cytokine release including TNF-α, IL-1β, and IL-6 [92]. Conversely, reduced calcium levels dampen immune cell activation, creating a physiological mechanism for controlling inflammatory intensity during metabolic stress [92].

This regulatory role of calcium, as described in [92], supports the hypothesis that hypocalcemia during transition periods is an adaptive strategy to prevent excessive immune activation, rather than solely a pathological condition requiring correction. During periparturient period, when tissue

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remodeling, microbial exposure, and metabolic challenges create inherent inflammatory risk, the organism strategically lowers circulating calcium to prevent inflammatory overactivation - essentially "turning down" the immune system's sensitivity to avoid collateral tissue damage while maintaining sufficient immune function for pathogen defense.

If validated, this concept necessitates fundamental reconceptualization of clinical approaches to hypocalcemia. Rather than reflexively supplementing calcium in all hypocalcemic animals, therapeutic decisions must account for inflammatory status: calcium supplementation in animals with active inflammation may be iatrogenic, activating immune responses at precisely the time when dampening would be protective. Preliminary analyses suggest that integrated assessment of calcium-inflammation interactions provides substantially greater predictive power for disease outcomes than either measurement alone [data not shown], supporting the existence of code-like information processing beyond simple biochemical correlations.

17.2. Clinical and Research Implications

The proposed **CKIC** operates through hierarchical information processing layers. At the molecular level, individual metabolites (BHB, NEFA, glucose) and cytokines function as elementary signals - the "vocabulary" of the code. However, their biological meaning emerges not from static concentrations but from temporal patterns: rates of change (first derivatives), acceleration (second derivatives), and trajectory curvature distinguish transient perturbations from sustained threats and predict whether systems will stabilize or progress toward pathology [39,40].

Critically, the same biochemical pattern generates different physiological outcomes depending on circumstances - the essence of code-dependent interpretation [41]. For example, BHB = 1.2 mmol/L coupled with low calcium and controlled inflammation may represent successful adaptive ketosis, whereas identical BHB levels with elevated calcium and rising inflammation signal impending metabolic decompensation. This context-dependency mirrors semantic properties of human language, where identical words convey different meanings in different contexts.

Information-theoretic analyses reveal that metabolic and inflammatory markers exhibit properties characteristic of sophisticated communication systems, including redundancy (multiple signals encoding similar information for error correction), synergistic information (combinations providing greater predictive power than individual measurements), and directed information flow (transfer entropy quantifying causal influences) [67,68]. The calcium-inflammation interaction appears to function as a regulatory "control bit" that modulates interpretation of metabolic signals, analogous to header information in digital communication protocols specifying how message content should be decoded [manuscript in preparation].

17.3. Broader Implications and Future Directions

Recognition of code-like properties in metabolic-immune integration has immediate clinical implications. Traditional diagnostic approaches relying on threshold-based classification of individual biomarkers (BHB >1.2 mmol/L for ketosis, Ca^{2+} <2.0 mmol/L for hypocalcemia) obscure the information content embedded in patterns and interactions. Code-based diagnosis requires integrated assessment: not "Is BHB elevated?" but rather "What pattern do BHB, calcium, and inflammatory markers form, and what does this pattern signify about future trajectories?"

Preliminary work developing machine learning algorithms to recognize and interpret these patterns demonstrates prediction of clinical ketosis with >90% accuracy at 14 days before diagnosis [14]; [manuscript in preparation] - substantially earlier and more accurate than conventional screening approaches. This predictive power derives from the recognition that current biochemical patterns encode information about future trajectories, analogous to how current weather patterns enable meteorological forecasting.

Therapeutically, code-based approaches shift focus from treating abnormal laboratory values to restoring system integrity. If inflammation is driving dysregulation, interventions targeting inflammatory reduction may prove more effective than metabolic support alone. If calcium-

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dependent immune gating is dysregulated, treatment strategies must account for this regulatory failure rather than simply supplementing deficient metabolites. The framework suggests that successful intervention requires understanding which aspect of code integrity has failed and targeting restoration of regulatory function rather than normalization of individual parameters.

17.4. Clinical Translation: From Code Comprehension to Therapeutic Application

The CKIC framework potentially extends beyond transition dairy cattle to any mammalian system navigating metabolic-immune stress. Similar regulatory challenges occur in human metabolic syndrome, fasting, lactation across species, and critical illness - situations where organisms must coordinate competing demands of energy mobilization and immune defense. The remarkable conservation of calcium signaling, ketone metabolism, and innate immunity across mammalian taxa suggests that this regulatory architecture may represent a fundamental biological organizing principle rather than a species-specific adaptation.

Testing this framework rigorously requires prospective validation studies examining whether code-based diagnostic and therapeutic approaches improve outcomes compared to conventional management. Such studies should assess not only predictive accuracy but also clinical utility: Do earlier predictions enable more effective interventions? Does code-guided therapy reduce disease incidence and severity? What are the economic implications of shifting from reactive disease treatment to predictive code-based management?

From a basic science perspective, critical questions remain regarding mechanisms of calcium-dependent immune regulation during metabolic stress, temporal dynamics of system responses, sources of individual variation in code integrity, and potential for epigenetic memory of prior code disruptions influencing future lactations. Investigation of these questions will require integration of metabolomics, immunology, systems biology, and information theory - truly interdisciplinary approaches matching the integrated nature of the biological system under study.

17.5. Evolutionary Perspective: The Code as Ancient Adaptive Language

The CKIC represents a conceptual evolution from viewing metabolism and immunity as separate systems that occasionally interact, toward recognizing them as components of an integrated information processing architecture. Calcium emerges not merely as a metabolite requiring homeostatic regulation but as a master regulatory signal that gates immune activation and thereby determines whether metabolic adaptations remain physiological or progress to disease. This code-based framework provides both deeper mechanistic understanding and enhanced clinical utility, enabling early prediction of disease trajectories and rational therapeutic decisions guided by underlying regulatory dysfunction rather than superficial laboratory abnormalities.

Perhaps most importantly, this framework exemplifies a broader paradigm shift occurring across biology and medicine: from reductionist approaches studying individual pathways in isolation toward systems-level recognition of integrated networks processing biological information. Health is not the absence of abnormal values but rather the maintenance of code integrity - the capacity to accurately encode, transmit, and decode physiological signals. Disease represents information processing failure - errors that prevent appropriate responses to environmental and internal challenges.

The detailed characterization of the CKIC, including comprehensive metabolomic validation, information-theoretic formalization, prospective clinical trials, and therapeutic protocols, will be presented in forthcoming dedicated publications. The present review establishes the conceptual foundation and synthesizes existing evidence supporting this integrative framework, pointing toward a new era of code-based precision medicine in which treatments are tailored not to population averages or simple laboratory thresholds but to individual animals' unique information processing patterns, physiological contexts, and regulatory capacities.

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