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Hypothesis

Committed Dietary Patterns and Mucosal Immune Tolerance: A Multimechanism Hypothesis with Bile Acid Signaling as a Testable Intermediate

Running Title: Dietary Commitment, Bile Acid Signaling, and Mucosal Immune Tolerance

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Summary

IBD and PSC patients share a microbiome depletion pattern that impairs mucosal immune tolerance. This paper proposes that committed dietary patterns restore this balance through multiple interdependent signaling networks, with bile acid signaling as the primary testable intermediate, and presents a falsifiable experimental program.

Abstract

Patients with inflammatory bowel disease (IBD) and primary sclerosing cholangitis (PSC) share a characteristic depletion of bile acid-transforming bacteria, undermining intestinal mucosal immune tolerance. These organisms convert lithocholic acid into immunomodulatory secondary bile acid species: 3-oxolithocholic acid, isoallolithocholic acid, and isolithocholic acid, which suppress Th17 differentiation and expand Foxp3$^+$ regulatory T cells through RORγt binding and mitochondrial signaling. This paper proposes that committed dietary patterns at either metabolic pole, verified nutritional ketosis or traditional Mediterranean diet, restore coherent signaling across multiple interdependent receptor and metabolic sensing networks (FXR, TGR5, S1PR2, RORγt, and an intersecting oxysterol-LXR axis) and, through that coherence, support mucosal immune tolerance. Bile acid signaling is the best-characterized candidate mechanism for one arm of this network and the primary testable intermediate the experimental program interrogates. Under committed ketosis, lipoprotein remodeling is most directly attributable to malonyl-CoA depletion and CPT-1 disinhibition; bile acid pool restructuring is a parallel candidate for immune effects. Mediterranean diet co-directional lipid and immune improvements are supported by IBD-specific randomized controlled trials. The equivalent prediction for committed ketosis, that both domains improve simultaneously in the same subjects under BHB-verified conditions, has not been demonstrated; IBD-specific clinical evidence for the ketogenic pole currently rests on a ten-patient case series, making it the primary hypothesis under experimental test rather than an established parallel. Intermediate carbohydrate restriction, defined by the absence of verified ketosis, is hypothesized to produce oscillating rather than coherent receptor engagement, producing neither lipid nor immune improvement reliably. No study has simultaneously characterized dietary metabolic state, the bile acid metabolome, and Th17/Treg balance in the same IBD patients. A staged experimental program is proposed; a cross-sectional design in quiescent IBD patients constitutes the immediate test. The framework is directly falsified if committed and intermediate dietary groups do not differ in species-level immunomodulatory bile acid concentrations despite differing in lipid and immune outcomes; such a result would redirect Stage 3 design toward BHB-direct NLRP3 inhibition and Kbhb-mediated mTOR signaling as the primary mechanistic candidates. The Host-Microbe Counter-Regulation Index (HMCRI) — a candidate index requiring empirical validation, with no currently established reference ranges — is proposed as a systems-level index of signaling coherence whose reference ranges and dietary responsiveness constitute primary Stage 1 analytical objectives.

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Keywords: bile acids; FXR; TGR5; gut microbiome; immune tolerance; Treg; inflammatory bowel disease; primary sclerosing cholangitis; oxysterols; ketogenic diet; Mediterranean diet

Introduction

1. The Mucosal Immune Problem: Why Th17/Treg Balance Matters in IBD

Crohn's disease and ulcerative colitis are characterized by a breakdown of mucosal immune tolerance, specifically a shift in the intestinal Th17/Treg balance toward pro-inflammatory Th17 dominance that sustains and amplifies epithelial injury. The success of anti-IL-12/23 therapy (ustekinumab) and IL-23–selective blockade (risankizumab, mirikizumab) in both conditions strongly implicates Th17 pathway activation as a proximate driver of pathological inflammation rather than an epiphenomenon [1–3]. The parallel failure of broader immune suppression strategies to induce durable remission in substantial patient fractions suggests that restoring tolerance mechanisms, rather than simply attenuating inflammatory effectors, is the more productive therapeutic aim.

Two convergent lines of evidence implicate the gut microbiome and its bile acid–transforming capacity as upstream regulators of this Th17/Treg imbalance. First, Paik et al. (2022) identified specific human gut bacteria (primarily Gordonibacter pamelaeae and related Coriobacteriaceae) and their 3α/3β-hydroxysteroid dehydrogenase enzyme pairs as responsible for biosynthetic conversion of lithocholic acid into 3-oxolithocholic acid (3-oxoLCA) and isolithocholic acid (isoLCA), demonstrated this pathway in human stool samples, and found that both metabolites and their biosynthetic genes were significantly depleted in Crohn's disease patients across two independent cohorts, with levels inversely correlating with IL-17-related host gene expression [4]; the statistical signal was driven predominantly by CD, with UC depletion emerging in secondary analyses of a smaller dysbiotic UC subset.

Second, fecal microbiome analysis in primary sclerosing cholangitis, an immune-mediated cholestatic cholangiopathy in which 50–80% of affected patients carry concurrent IBD, predominantly ulcerative colitis [79,84], and consistently demonstrates depletion of Eubacterium spp. and Ruminococcus obeum, genera encoding enzymatic machinery for secondary bile acid transformation, independent of IBD status [5]. Chan et al. (2024) also confirmed lower fecal deoxycholic acid correlating with Blautia and Lachnoclostridium in PSC patients [6]. Mousa et al. (2021), analyzing 400 PSC patients vs. 302 controls at Mayo Clinic, found significantly elevated primary-to-secondary bile acid ratios confirming deficient conversion at population scale [7].

Wang et al. (2025) demonstrated that isoalloLCA attenuates intestinal inflammation in pediatric UC patients by metabolically reprogramming macrophages from glycolysis toward oxidative phosphorylation through ETS2-HIF1A/PFKFB3 inhibition [80]. Separately, Kabil et al. (2025) identified an ILC3-selective role in intestinal fibrosis through RORγt-dependent signaling in CD11c+ myeloid cells [82], extending the relevance of this pathway beyond luminal inflammation to fibrotic complications of Crohn's disease. The mechanistic inference is well-supported but incomplete: depletion of the bacteria that produce immunomodulatory secondary bile acids plausibly removes a constitutive tolerogenic signal from the colonic mucosal environment, shifting the Th17/Treg equilibrium toward inflammation; the middle link in this chain, direct species-level metabolite measurement, remains unmeasured in PSC and IBD tissue. Whether dietary pattern influences this depletion, and whether restoring the bile acid signaling environment through dietary commitment could represent a tractable adjunctive strategy, is the question this paper addresses.

Three empirical patterns motivate the broader hypothesis. First, committed Mediterranean diet reduces cardiovascular events by approximately 30% with simultaneous improvements across lipid, inflammatory, and glycemic domains [8,9]. Second, carbohydrate restriction in a cohort managed toward nutritional ketosis produced an internally coherent metabolic profile including decreased small LDL particle number, reduced large VLDL particles, and improved inflammatory markers at

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one year [10,11]; the committed-ketosis threshold was not universally reached in that cohort, as discussed in Section 11. Third, strict ketogenic diet produces marked microbiome restructuring with near-complete Bifidobacterium depletion through substrate deprivation and BHB-mediated growth inhibition, with confirmed downstream small-intestinal Th17 cell reduction via fecal transplant [12]; whether this effect extends to colonic Th17 populations (the compartment most directly relevant to IBD) has not been established. The co-directional nature of these improvements (metabolic and immune outcomes moving together under dietary commitment) motivates the search for a shared upstream coordinating mechanism. That the improvements are co-directional rather than independent points toward a proposed coupled regulatory network in which bile acid signaling, oxysterol biology, microbiome ecology, and mucosal immune tone are mutually constraining and collectively responsive to dietary pattern.

Whether a shared upstream mechanism — bile acid signaling — coordinates the co-directional metabolic and immune improvements under committed dietary patterns, or is merely a correlate of those patterns, remains untested: no study has simultaneously verified metabolic state, characterized the bile acid metabolome, and measured Th17/Treg balance in the same subjects. This paper proposes bile acid signaling as a coordinating mechanism, not an established one, and offers a staged experimental program designed to test or falsify that claim.

Hypothesis Development

2. Bile Acid Signaling as the Proposed Mechanistic Link

Bile acids, once understood as passive fat-absorption facilitators, are now recognized as a multi-receptor signaling network influencing lipid metabolism, mucosal immunity, and systemic inflammatory tone. The gut microbiome generates the majority of bile acid structural diversity through bacterial deconjugation (BSH enzymes), 7α-dehydroxylation (Clostridium scindens, C. hylemonae), and further epimerization producing immunomodulatory species including 3-oxolithocholic acid, isoallolithocholic acid, and isodeoxycholic acid [18,19,20]. Germ-free animals lack secondary bile acids entirely, confirming the microbiome's obligate role [90,74]. Critically, Won et al. (2025) discovered that the host simultaneously produces bile acid–methylcysteamine (BA-MCY) conjugates via VNN1 that act as potent FXR antagonists, revealing a host–microbe metabolic counterbalance modulating bile acid homeostasis [21]. The bile acid signaling landscape is therefore shaped by both microbial transformation (generating FXR agonists) and host counter-regulation (generating FXR antagonists), with dietary pattern influencing the balance. Directly relevant to IBD, Won et al. (2025) found BA-MCY conjugates significantly elevated in DSS colitis [21]. Separately, bsh gene variants enriched in Crohn's disease alter inflammatory outcomes through bile acid conjugation and hydrolysis activity [81].

Four primary receptor systems transduce bile acid signals into metabolic and immune outputs across tissues. (VDR also responds to secondary bile acids, specifically lithocholic acid, with tolerogenic effects on dendritic cells, but its contribution is not mechanistically developed in the present framework.) Hepatic FXR (NR1H4) governs bile acid synthesis (CYP7A1/CYP8B1 repression via SHP), lipogenesis (SREBP-1c suppression), and lipoprotein remodeling. Intestinal FXR inhibition improves metabolic outcomes in obesity models, while hepatic FXR activation is protective; the same receptor produces opposing metabolic effects depending on tissue context [22,23]. FXR's metabolic and anti-inflammatory programs are mediated by distinct post-translational modifications: SUMO2 modification at K277 redirects individual FXR molecules from RXRα-dependent metabolic gene transactivation to NF-κB transrepression [24]. Both programs can coexist within the same cell; the SUMO2 switch therefore operates at the level of individual molecules rather than cell populations. TGR5 (GPBAR1) on enteroendocrine L-cells stimulates GLP-1 secretion, while TGR5 on macrophages drives cAMP/PKA-mediated NLRP3 phosphorylation (Ser291 in mouse; Ser295 in human ortholog), blocking inflammasome assembly [25]. TGR5 activation also reduces oxidized LDL uptake and macrophage lipid loading in atherosclerosis models [26], representing the clearest example of cell-

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autonomous coupling of metabolic and immune functions in this receptor class. Protein kinase D phosphorylates the same residue at the Golgi in an activating rather than inhibitory context [27], underscoring that the anti-inflammatory output of TGR5 is pathway- and compartment-dependent.

A third receptor, S1PR2, mediates hepatic lipid metabolism and immune cell trafficking: under dietary conditions, conjugated bile acids signal through hepatocyte S1PR2 via SphK2 to upregulate lipid metabolism genes [28], a metabolic-protective role distinct from S1PR2's pro-inflammatory behavior in pathological injury contexts via SphK1 in macrophages [29]; these are two mechanistically separate pathways detailed in Table 1.

The fourth pathway operates at the Th17/Treg interface directly. Microbially generated 3-oxolithocholic acid directly binds RORγt to suppress Th17 differentiation [18]. Isoallolithocholic acid promotes Foxp3+ Treg differentiation through mitochondrial reactive oxygen species signaling and the Foxp3 CNS3 enhancer [18]. Li et al. (2021) identified NR4A1 as the nuclear hormone receptor required for isoalloLCA-mediated Treg differentiation, operating through a mitochondrial ROS and chromatin remodeling mechanism at the Foxp3 locus, independently of both FXR and VDR [30]. Isolithocholic acid (isoLCA) is a structurally related but functionally distinct stereoisomer that also inhibits Th17 differentiation via RORγt binding; the human cohort evidence for both 3-oxoLCA and isoLCA depletion in Crohn's disease is described in the Introduction [4]. Wang et al. (2025) found fecal isoalloLCA significantly depleted in pediatric UC patients in proportion to disease severity, with ex vivo TNF-α suppression confirmed in PBMCs from pediatric CD patients; reduced Parabacteroides distasonis, Parabacteroides merdae, and Parabacteroides gordonii abundance was separately demonstrated in adult IBD patients from the PRISM cohort, extending the isoalloLCA evidence base across age groups and disease subtypes [80]. The nomenclature distinction matters: isoalloLCA (Hang et al. 2019 [18]) and isoLCA (Paik et al. 2022 [4]) are distinct bile acid stereoisomers with distinct immune mechanisms: the former promotes Treg differentiation via mitochondrial ROS and the latter suppresses Th17 differentiation via RORγt, and both contribute to the framework's predicted Th17/Treg balance.

Isodeoxycholic acid illustrates the network's cell-type-dependent complexity. Campbell et al. (2020) demonstrated that isoDCA promotes peripheral Treg generation by acting as a functional antagonist of FXR on dendritic cells, though Campbell et al. themselves noted that additional FXR-independent pathways contribute and transcriptional changes persist in FXR-deficient DCs [19]. This cell-type specificity matters: Dong et al. (PNAS 2024) showed potent FXR agonism by isoDCA in HEK293 cells cotransfected with FXR/RXR, with activity at low micromolar concentrations suppressing Wnt signaling and colorectal cancer cell growth [31], illustrating the network's inherent capacity for opposing outputs depending on co-regulator context. A microbiome-responsive RORγt$^+$ antigen-presenting cell population ('Thetis cells') independently induces peripheral Treg differentiation via MHCII and ITGB8-mediated TGFβ1 activation [32,95,96,97], reinforcing that tolerogenic signaling at the mucosal interface operates through redundant, parallel mechanisms beyond the bile acid pathways described.

Bile acid–mediated immune tolerance carries an important constraint in oncological contexts. Varanasi et al. (2025) demonstrated that primary bile acids, principally taurochenodeoxycholic acid (TCDCA), impair CD8+ T cell function in hepatocellular carcinoma through oxidative stress, while LCA (and isoalloLCA) also operates through ER stress via ATF6, IRE1α, and PERK signaling, and that blocking BAAT enhanced anti-PD-1 immunotherapy [33]. Of note, UDCA was protective, enhancing rather than suppressing T cell function. This means the same bile acid signaling that promotes immune tolerance via Treg expansion and Th17 suppression may simultaneously suppress beneficial anti-tumor immunity depending on which bile acid species predominate. The framework proposed here applies to metabolic and autoimmune contexts; its extension to oncology requires additional consideration of this trade-off. Within the metabolic and autoimmune contexts that define this paper's scope, the four receptor systems described above (FXR, TGR5, S1PR2, and RORγt, with the oxysterol-LXR axis as an intersecting regulatory layer) constitute the mechanistic scaffold through

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which dietary commitment is proposed to generate coherent immune and metabolic outcomes. (Figure 1)

Table 1. Receptor systems mediating bile acid and oxysterol signals across metabolic and immune domains.

Receptor	Primary Dietary Ligands	Tissue / Cell Type	Key Downstream Pathway	Supporting Evidence	Predicted Dietary Effect	Key Ref(s)
FXR (NR1H4) — FARNESOID X RECEPTOR						
FXR (hepatic)	CDCA, DCA, LCA (secondary bile acids)	Hepatocytes	CYP7A1/CYP8B1 repression via SHP → bile acid synthesis suppression; SREBP-1c suppression → reduced lipogenesis; SUMO2-K277 → NF-κB transrepression	OCA (REGENERATE) improved hepatic fibrosis despite atherogenic lipid shift — confirms hepatic FXR engagement at pharmacological concentrations [39]; DIRECT-PLUS [44]: baseline fecal BAs modified MedDiet cardiometabolic response	**KD:** elevated secondary BAs activate hepatic FXR; suppresses CYP7A1, reduces VLDL production **MedDiet:** diverse secondary BA pool maintains hepatic FXR tone **Intermediate zone:** oscillating BA environment — predicted incomplete FXR activation	[22,23,24]
FXR (intestinal)	Secondary bile acids (same ligands; opposing functional context to hepatic FXR)	Intestinal epithelial cells	Intestinal FXR inhibition improves metabolic outcomes in obesity models; FGF15/19 signaling to liver; psyllium → intestinal FXR activation → colitis protection	Bretin et al. 2023 [49]: psyllium protects against DSS and T-cell-transfer colitis through FXR activation; first direct IBD-relevant demonstration of dietary fiber acting	**KD:** substrate-depleted microbiome alters secondary BA profile at intestinal epithelium **MedDiet:** psyllium and soluble fiber components activate intestinal	[22,23,49]

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Receptor	Primary Dietary Ligands	Tissue / Cell Type	Key Downstream Pathway	Supporting Evidence	Predicted Dietary Effect	Key Ref(s)
			abolished in FXR-KO mice	via intestinal FXR	FXR; Bretin 2023 confirms FXR-dependent colitis protection	
TGR5 (GPBAR1) — G PROTEIN-COUPLED BILE ACID RECEPTOR						
TGR5 (L-cell)	LCA, DCA, TDCA, TUDCA (secondary and taurine-conjugated species)	Enteroendocrine L-cells (ileum and colon)	cAMP → GLP-1 secretion → hepatic lipogenesis suppression, insulin sensitization, innate immune modulation; GLP-1 analogue therapy reduces inflammatory cytokines in immune cells [72]	TDCA and TUDCA elevated in KD-fed mice; correlational support n=416 observational and n=25 interventional human cohorts (Li et al. [50]: RED — no BHB verification; admissible as mechanistic plausibility for BSH-TGR5 pathway)	**KD:** elevated TDCA/TUDCA provide TGR5 agonism; predicted enhanced GLP-1 output **MedDiet:** diverse secondary BA pool maintains TGR5 stimulation **Intermediate zone:** oscillating environment — predicted incomplete TGR5 activation	[25,26,50,72]
TGR5 (macrophage)	LCA, DCA, TLCA (potent agonists)	Macrophages (intestinal and systemic)	cAMP/PKA → NLRP3 phosphorylation (Ser291 mouse; Ser295 human ortholog) → inflammasome assembly blocked [25]; PKD phosphorylates same residue at Golgi in	TGR5 activation reduces oxidized LDL uptake and macrophage lipid loading in atherosclerosis models [26]— clearest example of cell-autonomous	**Both committed patterns:** secondary BAs maintain TGR5-mediated NLRP3 suppression **Intermediate zone:** oscillating BA environment	[25,26,27]

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Receptor	Primary Dietary Ligands	Tissue / Cell Type	Key Downstream Pathway	Supporting Evidence	Predicted Dietary Effect	Key Ref(s)
			activating context [27] — anti-inflammatory output is pathway- and compartment-dependent	metabolic-immune coupling in this receptor class	— predicted incomplete inflammasome suppression	
S1PR2 — SPHINGOSINE-1-PHOSPHATE RECEPTOR 2						
S1PR2 (hepatocyte, dietary context)	Conjugated bile acids (taurine- and glycine-conjugated primary and secondary species)	Hepatocytes	SphK2 → nuclear S1P → HDAC1/2 inhibition → SREBP-1c, FAS, LDLR upregulation → lipid metabolism gene activation [28]	Metabolic-protective role under dietary bile acid signaling [28]; distinct from injury-context S1PR2 behavior (see row below; see footnote[1])	**KD:** elevated TDCA/TUDCA modulate hepatocyte S1PR2 tone **MedDiet:** diverse conjugated pool; S1PR2 hepatic signaling maintained	[28]
S1PR2 (macrophage, injury context — see footnote[1])	Sphingosine-1-phosphate (S1P) — not a bile acid ligand in this context	Infiltrating macrophages (pathological liver injury)	SphK1 → NLRP3 inflammasome priming → pro-inflammatory cytokine expression [29]	Atorvastatin RCTs in UC [36–38]: serum S1P significantly reduced alongside IL-6 and TNF-α, linking HMG-CoA reductase inhibition to S1PR2/SphK1 pathway in colonic macrophages; included for disambiguation only Injury-context findings do not	Injury-context row; no dietary prediction applicable. Included to make the S1PR2 disambiguation visible at the table level.	[29,36–38]

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Receptor	Primary Dietary Ligands	Tissue / Cell Type	Key Downstream Pathway	Supporting Evidence	Predicted Dietary Effect	Key Ref(s)
				extrapolate to dietary signaling physiology		
RORΓT — RAR-RELATED ORPHAN RECEPTOR GAMMA T						
RORγt (colonic Th17 cell)	3-oxolithocholic acid (3-oxoLCA), isolithocholic acid (isoLCA) — direct binding; competitive RORγt antagonists; 27-OHC (oxysterol) is a RORγt agonist in functional antagonism	Colonic Th17 cells	3-oxoLCA and isoLCA bind RORγt → suppress Th17 differentiation [18]; 27-OHC activates RORγt → promotes Th17, suppresses Tregs [64,65] — functional antagonism at same receptor	Paik et al. 2022 [4]: 3-oxoLCA and isoLCA depleted in CD patients across two independent cohorts; depletion inversely correlated with IL-17-related gene expression Wang et al. 2025 [80]: isoalloLCA depleted in pediatric UC in proportion to disease severity	**KD:** predicted increased 3-oxoLCA/isoLCA availability under committed microbiome configuration; 27-OHC production may decrease if hepatic cholesterol synthesis reduced (speculative) **MedDiet:** fiber substrate maintains Coriobacteriaceae; sustained 3-oxoLCA/isoLCA production **Intermediate zone:** incomplete microbiome restructuring — insufficient or oscillating 3-oxoLCA/isoLCA	[4,18,64,65,80]

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Receptor	Primary Dietary Ligands	Tissue / Cell Type	Key Downstream Pathway	Supporting Evidence	Predicted Dietary Effect	Key Ref(s)
RORγt (Foxp3⁺ Treg / isoalloLCA axis — see footnote [2])	Isoallolithocholic acid (isoalloLCA) — distinct stereoisomer from isoLCA; see footnote [2]	Foxp3⁺ regulatory T cells (colonic)	isoalloLCA → mitochondrial ROS → Foxp3 CNS3 enhancer → Foxp3⁺ Treg differentiation [18]; NR4A1 identified as downstream effector via distinct bacterial metabolite, independently of FXR and VDR [30]	Hirschberger et al. 2021 [87]: BHB-verified (self-managed ad libitum KD after counseling; BHB ≥0.5 mM confirmed at days 7, 14, 21), 44 healthy volunteers — Foxp3⁺ Treg expansion by flow cytometry, IL-10 upregulation, RNAseq-confirmed immunometabolic reprogramming (3 weeks; below 8-week threshold) Kabil et al. 2025 [82]: ILC3 suppression via RORγt-dependent mechanism attenuates intestinal fibrosis	**KD:** committed microbiome may reduce isoalloLCA-producing Coriobacteriaceae; Treg-expanding signal may be partially replaced by BHB-direct immunometabolic reprogramming (Hirschberger [87]) **MedDiet:** high-fiber substrate maintains Coriobacteriaceae; sustained isoalloLCA production and Foxp3⁺ Treg expansion	[18,30,82,87]
LXR (NR1H3/NR1H2) — LIVER X RECEPTOR (OXYSTEROL–STEROL IMMUNE AXIS)						
LXR-α (CD11c⁺ myeloid,	Oxysterols: 27-hydroxycholesterol, 25-	CD11c⁺ myeloid cells (mesenteric	LXRα deficiency increases mesenteric Th17 cells via isoform-	Parigi et al. 2021 [62]: LXRα deficiency increases mesenteric	**KD:** sustained malonyl-CoA depletion and β-oxidation	[62,63,64,65,66,67,68]

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Receptor	Primary Dietary Ligands	Tissue / Cell Type	Key Downstream Pathway	Supporting Evidence	Predicted Dietary Effect	Key Ref(s)
mesenteric)	hydroxycholesterol (derived from cholesterol via CYP27A1/CYP7B1)	lymph node)	specific mechanism [62]; Jacobse et al. [63]: IL-23R signaling downregulates LXR target genes in colonic Tregs → impairs Treg stability	Th17 cells specifically; LXRβ deficiency increases RORγt⁺ Tregs specifically through CD11c⁺ myeloid signaling (non-overlapping isoform-specific functions); human scRNA-seq confirms IL-23R on colonic Tregs [63]	predicted to reduce de novo cholesterol synthesis; potential 27-OHC reduction in macrophages (speculative; no direct human data) **MedDiet:** oxysterol remodeling not directly characterized under MedDiet No study has measured oxysterol profiles under committed dietary patterns in human IBD patients.	
LXR-β (CD11c⁺ myeloid, mesenteric)	Same oxysterol ligands as LXRα; broader tissue distribution	CD11c⁺ myeloid cells; also intestinal epithelium	LXRα deficiency increases mesenteric Th17 cells specifically; LXRβ deficiency increases RORγt⁺ Tregs specifically through CD11c⁺ myeloid signaling [62]; FXR-LXR crosstalk at	Parigi et al. 2021 [62]: LXRα-null mice show increased Th17 cells; LXRβ-null mice show increased RORγt⁺ Tregs — non-overlapping isoform-specific functions with distinct	**Both LXR isoforms:** framework proposes committed dietary patterns shift hepatic cholesterol flux, altering both the bile acid pool and oxysterol landscape simultaneously, engaging FXR, TGR5,	[62,63]

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Receptor	Primary Dietary Ligands	Tissue / Cell Type	Key Downstream Pathway	Supporting Evidence	Predicted Dietary Effect	Key Ref(s)
			shared gene regulatory networks predicted when bile acid and oxysterol precursor flux both altered	downstream cell types	LXRα, and LXRβ as co-equal nodes in the coupled network All LXR dietary predictions are speculative; no direct measurement in human IBD under committed dietary conditions	

[1] S1PR2 dietary (hepatocyte) and injury-context (macrophage) rows represent distinct signaling pathways through distinct kinases (SphK2 vs. SphK1) and must not be conflated. The injury-context row is included exclusively to make this disambiguation visible at the table level. [2] isoalloLCA (Hang et al. 2019 [18]) and isoLCA (Paik et al. 2022 [4]) are distinct bile acid stereoisomers with distinct immune mechanisms: isoalloLCA promotes Foxp3⁺ Treg differentiation via mitochondrial ROS; isoLCA suppresses Th17 differentiation via RORγt binding. [3] All entries in the Predicted Dietary Effect column are mechanistic inferences from receptor pharmacology and preclinical evidence — not direct measurements under committed dietary conditions in human IBD populations.

3. Primary Sclerosing Cholangitis as a Disease Model for Bile Acid–Immune Axis Disruption

Primary sclerosing cholangitis, a progressive fibro-inflammatory cholangiopathy in which IBD, predominantly ulcerative colitis, affects 50–80% of patients [79,84] (rates up to 88% are reported in cohorts using systematic endoscopic screening to detect subclinical disease), provides a naturally occurring human model of the bile acid signaling–immune tolerance breakdown this framework proposes. In PSC, fibro-inflammatory biliary stricturing disrupts the enterohepatic circulation, reducing colonic exposure to secondary bile acids and impairing the microbiome's capacity to generate immunomodulatory trace species. Kummen et al. (2021) demonstrated by shotgun metagenomics across German and Norwegian centers that PSC patients show depletion of Eubacterium spp. and Ruminococcus obeum, genera encoding the 3α/3β-HSDH and 7α-dehydroxylation enzymes required for 3-oxoLCA and isoLCA biosynthesis, independent of concurrent IBD status [5]. Chan et al. (2024) confirmed lower fecal deoxycholic acid in PSC vs. controls correlating with Blautia and Lachnoclostridium abundance, and Mousa et al. (2021) documented elevated primary-to-secondary bile acid ratios at population scale in 400 PSC patients, providing quantitative confirmation of deficient secondary bile acid conversion [6,7].

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The immunological consequences are consistent with predicted downstream effects of this bile acid depletion. Poch et al. (2021), using single-cell RNA sequencing and ATAC-seq of intrahepatic T cells, found that PSC-expanded naive-like CD4+ T cells had the highest trajectory probability (0.57) of differentiating toward Th17 rather than Foxp3+Treg, with chromatin accessibility confirming Th17-biased imprinting [34]. Shaw et al. (2023, Nature Medicine) identified a pathogenic IL-17A+FoxP3+ CD4+ T cell population enriched in PSC colon tissue that was significantly less frequent in patients with IBD without concurrent PSC (P = 0.024) and was associated with dysplasia risk [35]. These findings are mechanistically consistent with a deficit in 3-oxoLCA and isoLCA, whose RORγt-binding activity would be expected to suppress precisely this Th17-biased differentiation program. The causal contribution of specific gut pathobionts to hepatic Th17 responses has been demonstrated in a gnotobiotic mouse model: Nakamoto et al. (2019) showed that PSC-derived Klebsiella pneumoniae transferred to gnotobiotic mice produced Th17 responses and hepatobiliary injury, reversed by targeted antibiotic treatment [76].

Critically, no published study has directly measured 3-oxoLCA, isoalloLCA, isoLCA, or isoDCA in PSC stool or tissue. The inference chain (depleted secondary bile acid–producing bacteria → reduced trace immunomodulatory bile acids → Th17/Treg imbalance) is compelling, but the middle link remains an untested prediction. This gap (verified ketosis, simultaneous multi-domain characterization, ≥12 weeks) is one this framework identifies as a high-priority measurement target in the Stage 1 experimental design described below.

Clinical evidence that pharmacological targeting of the cholesterol synthesis pathway alters IBD-relevant inflammatory signals at clinically meaningful magnitudes is presented in Section 7 (Table 2).

Table 2. Human clinical evidence: bile acid, microbiome, and immune outcomes under dietary and pharmacological interventions.

Study / Year	Design	Population (n)	Intervention or Exposure	Bile Acid or Microbiome Outcome	Immune or Clinical Outcome
IBD AND PSC HUMAN COHORT DATA — BILE ACID AND MICROBIOME EVIDENCE					
Paik et al. *Nature 2022*	Cross-sectional; two independent IBD cohorts	CD patients and healthy controls	Gordonibacter pamelaeae and Coriobacteriaceae abundance; 3α/3β-HSDH enzyme gene expression	3-oxoLCA and isoLCA biosynthetic genes and fecal metabolite levels significantly depleted in CD across both cohorts vs. controls	Depletion inversely correlated with IL-17-related host gene expression; most direct human translational evidence for the bile acid–Th17 regulatory axis in IBD [4]
Kummen et al. *Gastroenterology 2021*	Shotgun metagenomics; multicenter	PSC patients and controls; n≈300	PSC microbiome composition; stratified by concurrent IBD status	Eubacterium spp. and Ruminococcus obeum depleted in PSC independent of IBD status; genera encode 3α/3β-HSDH and 7α-dehydroxylation	IBD-independent depletion: bile acid-transforming capacity impaired upstream of diet-microbiome interaction; establishes PSC as

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Study / Year	Design	Population (n)	Intervention or Exposure	Bile Acid or Microbiome Outcome	Immune or Clinical Outcome
		combined		enzymes for 3-oxoLCA/isoLCA biosynthesis	human model of the proposed bile acid–immune tolerance breakdown [5]
Mousa et al. *Hepatology 2021*	Population-scale cross-sectional; Mayo Clinic	400 PSC vs. 302 controls	Fecal and serum bile acid profiles; primary-to-secondary bile acid ratios	Markedly elevated primary-to-secondary bile acid ratios in PSC; quantitative confirmation of deficient secondary bile acid conversion at population scale	Quantitative confirmation of the bile acid environment predicted by microbiome depletion findings; strengthens PSC-as-model-disease argument [7]
Chan et al. *JHEP Rep 2024*	Cross-sectional with 16S microbiome profiling	26 early-stage PSC patients and controls	Fecal bile acids; gut microbiota; dietary intake; BA synthesis and FXR activity markers	Fecal DCA significantly lower in PSC vs. controls (p_{aej}=0.04); Blautia and Lachnoclostridium abundance positively correlated with fecal DCA; DCA negatively correlated with total bilirubin (p=0.006)	DCA reduction not mediated by BA synthesis or FXR activation; microbiome-driven mechanism; Blautia and Lachnoclostridium as candidate restorative genera under dietary intervention [6]
⚠ **MEASUREMENT GAP** — No published study has directly measured 3-oxoLCA, isoalloLCA, isoLCA, or isoDCA in PSC stool or tissue. The inference chain (depleted secondary BA-producing bacteria → reduced immunomodulatory trace bile acids → Th17/Treg imbalance) is the central untested prediction of this framework. Direct trace bile acid measurement in PSC stool is a Stage 1 priority target.					
MEDITERRANEAN DIET — RCT AND COHORT EVIDENCE IN IBD-RELEVANT POPULATIONS					
Seethaler et al. *Life Sci 2025*	Exploratory analysis of RCT (LIBRE trial; NCT02087592)	68 women with impaired intestinal barrier (n=33 Med Diet,	Mediterranean diet vs. standard diet; 12 weeks	MedDiet decreased fecal DCA and LCA; increased UDCA; formal mediation analysis confirmed bile acid changes mediated beneficial effects on intestinal barrier integrity (zonulin, LBP)	First RCT demonstrating that MedDiet-induced bile acid compositional shifts mechanistically mediate a gut-specific endpoint; distinct from DIRECT-PLUS (baseline BAs modified response magnitude) [45]

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Study / Year	Design	Population (n)	Intervention or Exposure	Bile Acid or Microbiome Outcome	Immune or Clinical Outcome
		n=35 control)			
Strauss, Haskey et al. *Int J Mol Sci 2023*	WGCNA metabolomics; randomized pilot (NCT04474561; small n[1])	n=29 quiescent UC; Med Diet n=13 responders, n=16 non-responders	Mediterranean diet vs. Canadian habitual diet; 12 weeks	Bile acid profiles within a WGCNA-identified metabolite cluster mediated the relationship between Mediterranean diet score and fecal calprotectin	Pilot-scale signal for bile acid-mediated immunological response; Faecalibacterium prausnitzii, Dorea longicatena, Roseburia inulinivorans identified as functional mediators [47]
Haskey et al. *J Crohns Colitis 2023*	Randomized controlled trial (NCT03053713; pilot study[1])	n=28 quiescent UC; 12 weeks	Mediterranean diet vs. control diet; 12 weeks	Microbiome reshaped toward Mediterranean-associated taxa; dysbiosis markers improved in MedDiet arm; no direct bile acid metabolomics	20% of MedDiet participants had fecal calprotectin >100 µg/g vs. 75% of controls; IBD-specific RCT-level evidence for MedDiet effect on mucosal inflammatory activity [46]
Godny et al. *Gastroenterology 2025*	Prospective cohort; dietary recall-based adherence scoring (no dietary assignment)	271 newly diagnosed CD patients; median 27-month follow-up	Mediterranean diet adherence score; bile acid profiles, kynurenines, Faecalibacterium, SCFAs	Higher MedDiet adherence inversely correlated with primary bile acids and pro-inflammatory kynurenines; positively correlated with Faecalibacterium and SCFAs	MedDiet adherence inversely correlated with CDAI, fecal calprotectin, and CRP; largest IBD-specific cohort with simultaneous favorable shifts in lipid-adjacent and immune markers [48]

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Study / Year	Design	Population (n)	Intervention or Exposure	Bile Acid or Microbiome Outcome	Immune or Clinical Outcome
DIRECT-PLUS Trial (Gao P et al.) *Gut Microbes 2024*	Multi-omics RCT analysis; n=284	284 adults; healthy dietary guidelines or two Med Diet variants; 18-month follow-up	Longitudinal fecal bile acid metabolomics (44 species); gut microbiome shotgun sequencing	Baseline fecal BA levels significantly modified cardiometabolic response to MedDiet; 14 fecal BAs prospectively associated with BMI and lipid profiles	First RCT evidence of bile acid profile-mediated modification of a dietary intervention's cardiometabolic effect; mechanistically distinct from Seethaler 2025 [44]
KETOGENIC DIET — PRECLINICAL AND IBD-RELEVANT EVIDENCE					
Hirschberger et al. *EMBO Mol Med 2021*	Prospective intervention; BHB-verified (TIER 1)	44 healthy volunteers; ≤30g/day CHO; BHB verified ≥0.5 mM throughout	Strictly enforced very-low-carbohydrate diet; blood BHB verified ≥0.5 mM throughout; 3 weeks	No bile acid metabolomics conducted	Foxp3⁺ Treg expansion by flow cytometry; IL-10 upregulation; RNAseq-confirmed immunometabolic reprogramming toward oxidative phosphorylation. Only human study with BHB-verified committed ketosis demonstrating Treg expansion; 3 weeks (below 8-week threshold); healthy volunteers, not IBD patients [87]
Westman et al. *Int J Cardiol 2006*	24-week RCT vs. low-fat diet; urinar	119 overweight hyperlipidemic adult	Low-carbohydrate ketogenic program (≤20g/day CHO) vs.	Urinary ketones positive throughout; blood BHB not measured — committed-ketosis threshold unconfirmed (TIER 2)	KD arm: large VLDL −78%, small LDL −78%, medium LDL −42%, large HDL +21% by NMR; most detailed lipoprotein subfraction data under strict

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Study / Year	Design	Population (n)	Intervention or Exposure	Bile Acid or Microbiome Outcome	Immune or Clinical Outcome
	y ketone monitoring (TIER 2 — blood BHB not measured[2])	s (n=60 KD, n=59 low-fat); NMR lipoprotein subfraction analysis	low-fat diet		carbohydrate restriction; no immune outcomes measured [86]
Norwitz and Soto-Mota *Front Nutr 2024*	Case series (AMBER — signal only; no BHB documentation in C-11 format[3])	10 IBD patients (6 UC, 4 CD)	Carnivore-ketogenic diet; clinical outcomes reported; no BHB verification	No bile acid or microbiome measurements; metabolic state not verified by blood BHB meeting C-11 criteria	Universal clinical improvement reported; most discontinued medications; only IBD-specific human clinical signal for the ketogenic pole. Insufficient for clinical inference; provides signal for prospective investigation [54]
Kong et al. *Signal Transduct Target Ther 2021*	Mouse DSS colitis model (RED — murine; 6-hydroxylated BA pool absent in humans)	C57BL/6 mice; DSS colitis model	Ketogenic diet vs. control; fecal microbiota transfer to germ-free mice	KD restructured gut microbiota; fecal microbiota transfer confirmed microbiome-dependent mechanism	KD reduced colonic RORγt⁺CD3⁾ ILC3s and inflammatory cytokines; ILC3 reduction warrants prospective human investigation given ILC3 role in sustaining colonic Th17 programs in IBD [53]

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Study / Year	Design	Population (n)	Intervention or Exposure	Bile Acid or Microbiome Outcome	Immune or Clinical Outcome
Huang et al. *BMC Med* 2022	Human tissue + murine DSS model (GREEN for IBD relevance of BHB deficit; murine intervention component RED)	IBD patients and healthy controls (tissue); C57BL/6 mice (DSS model)	Colonic mucosal BHB measurement in IBD patients; rectal BHB enema in DSS colitis mice	BHB significantly reduced in colonic mucosa of UC and CD patients; inversely correlated with disease activity	Colonic mucosal BHB deficit in IBD patients is the primary IBD-specific translational finding (GREEN); STAT6/M2 mechanism in mice requires human validation; colonic BHB deficit is distinct from measuring dietary ketosis [52]
⚠ **MEASUREMENT GAP** — No study has simultaneously verified blood BHB ≥0.5 mM, characterized the bile acid and SCFA metabolome, and measured Th17/Treg balance in the same subjects under committed ketosis sustained ≥12 weeks. Hirschberger [87]: BHB-verified and Treg measured, but healthy volunteers, 3 weeks, no bile acid metabolomics. Westman [86]: lipoprotein subfractions documented (urinary ketone compliance only; blood BHB not measured; omega-3 supplementation confound) but no immune outcomes. This three-failure design gap is the primary methodological motivation for the Stage 1 experimental program.					
CHOLESTEROL–IMMUNE NODE — STATIN RCT EVIDENCE IN UC (INDIRECT SUPPORT FOR OXYSTEROL-LXR AXIS)					
AlRasheed, Alarfaj, Khrieba et al. *J Clin Med / Front Med / Front Pharmacol* 2025	Three independent RCTs (same clinical program, Tanta and Horus	Active UC; combined n≈300; atorvastatin 80mg added to standard	Atorvastatin adjunctive therapy vs. mesalamine alone; multiple biomarker panels	Alarfaj et al. [37]: significant reduction in serum S1P in atorvastatin arm — links HMG-CoA reductase inhibition to S1PR2/SphK1 pathway in colonic macrophages; Mendelian randomization [69]: HMGCR-mediated LDL-C lowering does not increase	All three trials: significant reductions in disease activity, IL-6, TNF-α, and fecal calprotectin vs. mesalamine alone; converging evidence that cholesterol pathway interventions alter IBD-relevant inflammatory signals through pleiotropic

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Study / Year	Design	Population (n)	Intervention or Exposure	Bile Acid or Microbiome Outcome	Immune or Clinical Outcome
	University)	mesalamine		IBD risk; PCSK9-mediated LDL-C lowering paradoxically does	mechanisms [36–38,69]

[1] Haskey 2023 [46] (NCT03053713) and Strauss/Haskey 2023 [47] (NCT04474561) were pilot studies; results require confirmatory replication in adequately powered cohorts. [2] Westman [86] is TIER 2 under C-11: urinary ketone monitoring only; blood BHB not measured; committed-ketosis threshold unconfirmed. [3] Norwitz and Soto-Mota [54] is AMBER: case series; no BHB documentation in C-11 format; insufficient for clinical inference. RED entries (Kong [53], Huang [52] murine intervention) derive from DSS mouse models with 6-hydroxylated BA pools absent in humans; mechanistic inferences require prospective human validation. The Soto-Mota et al. 2025 longitudinal KETO-CTA study [16] was retracted by JACC Advances; no reliable longitudinal plaque data are available from that cohort. TIER 1 classification for Burén 2021 [88] reflects blood BHB ≥0.5 mM confirmed in every participant at study endpoint (day 29) with daily compliance monitored by urinary ketones; it does not reflect continuous blood BHB verification throughout the study.

Mechanistic Framework

4. The OCA Dissociation and Bile Acid Sequestrant Evidence

The pharmacological record of FXR agonism provides a natural experiment in what happens when one node of the combined signaling network is activated in isolation at sustained supraphysiological concentrations. In the REGENERATE Phase III trial (n=931), obeticholic acid achieved its primary endpoint of statistically significant hepatic fibrosis improvement (22.4% vs. 9.6%, $p<0.0001$) while simultaneously producing an atherogenic lipid shift [39]; the concurrent deterioration in the lipid domain, even as the hepatic inflammatory domain improved, is precisely what a multi-receptor framework predicts when signaling coherence across the full receptor network is not established. Regulatory rejection reflected an unfavorable benefit-risk profile: the atherogenic lipid shift, pruritus burden (likely attributable to off-target TGR5/MRGPRX4 activation rather than FXR-related toxicity), and uncertain long-term outcomes; this does not reflect the absence of pharmacodynamic FXR engagement. The FDA issued a Complete Response Letter in June 2023 for the NASH indication, and Intercept subsequently abandoned all NASH development. The EMA revoked Ocaliva's conditional marketing authorization for primary biliary cholangitis in August 2024 following the failed COBALT confirmatory trial (HR 1.01, 95% CI 0.68–1.51) [40]; the FDA withdrew approval of the PBC indication in November 2025 after a 13-to-1 advisory committee vote finding that clinical benefit could not be verified, and a subsequent 10-to-1 benefit-risk vote; Intercept voluntarily withdrew the marketing authorization following FDA notification of intent to withdraw approval. Obeticholic acid no longer holds a marketing authorization in any jurisdiction. No next-generation FXR agonist has resolved the atherogenic dyslipidemia that appears to be a class effect; resmetirom (a THR-β agonist, not an FXR agonist) was approved for MASH in March 2024 and received European Commission authorization in August 2025, effectively supplanting the FXR agonist class for this indication [41]. INT-767, the first dual FXR/TGR5 agonist to enter human trials (Phase 1, initiated 2015), was discontinued without published results; a second dual agonist, BAR502, has since entered Phase 1 evaluation (NCT05203367) but has not reported clinical outcomes.

Conversely, bile acid sequestrants provide indirect evidence that modifying the bile acid signaling environment can improve cardiovascular outcomes. The LRC-CPPT (n=3,806; 7.4-year follow-up) demonstrated 19% relative CHD risk reduction with cholestyramine [42]. However, absolute risk reduction was 1.6 percentage points, statistical significance relied on a one-tailed test, and all-cause mortality was not reduced. The LRC-CPPT's principal contemporary relevance lies not

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in its cardiovascular outcome data but in its mechanistic implication: bile acid pool modification through pharmacological sequestration alters the same enterohepatic circuit that this framework proposes to be modifiable by dietary commitment, a parallel that, while speculative, is directly testable in the Stage 1 design.

5. Two Committed Dietary Configurations and Their Bile Acid Environments

5.1. Mediterranean Configuration

High-fiber Mediterranean diet sustains saccharolytic fermenters producing millimolar colonic butyrate, which activates GPR109A on colonocytes and macrophages, indirectly supporting Foxp3+ Treg expansion [43]. The diverse microbiome generates secondary bile acids including isoDCA, which promotes Treg differentiation via FXR antagonism on dendritic cells [19]. The DIRECT-PLUS trial (Gao et al., n=284) demonstrated that baseline fecal bile acid levels significantly modified the cardiometabolic response to Mediterranean diet; this was the first RCT evidence of bile acid profile-mediated modification of a dietary intervention's effect [44].

New RCT-level evidence specifically in IBD populations strengthens this picture. Seethaler et al. (2025, LIBRE-1 RCT, n=68 women with impaired intestinal barrier) demonstrated that Mediterranean diet decreased fecal deoxycholic acid and lithocholic acid while increasing UDCA, and that formal mediation analysis confirmed bile acid changes mediated beneficial effects on intestinal barrier integrity [45]. This is the first RCT demonstration that Mediterranean diet-induced bile acid compositional shifts mechanistically mediate a gut-specific endpoint; this is distinct from the DIRECT-PLUS finding, in which baseline bile acid levels modified the magnitude of cardiometabolic response [44]. Haskey et al. (2023, n=28 quiescent UC, randomized; a pilot study requiring confirmatory replication) found that 20% of Mediterranean diet participants had fecal calprotectin >100 μg/g vs. 75% of controls after 12 weeks, with bile acid profiles within a WGCNA-identified metabolite cluster mediating the relationship between diet and calprotectin [46,47]. Haskey et al. (2025) subsequently characterized the metabolomic signatures of Mediterranean diet response in UC, identifying fiber-degrading Bacteroides species as key mediators and documenting bile acid shifts associated with dietary adherence [102]. Godny et al. (2025, prospective cohort, n=271 newly diagnosed CD patients) found MedDiet adherence inversely correlated with primary bile acids and pro-inflammatory kynurenines, while correlating positively with Faecalibacterium and SCFAs, and inversely with CDAI, fecal calprotectin, and CRP [48].

In a prospective cohort of 693 IBD outpatients (median 27 months), adherence to a healthy lifestyle combining Mediterranean diet and physical activity was associated with a 75% reduction in moderate-to-severe relapse risk (aHR 0.250, 95% CI 0.093–0.670) [83]; Mediterranean diet adherence alone did not reach significance for severe relapse endpoints in the separate analysis. Bretin et al. (2023) demonstrated that psyllium fiber, a characteristic Mediterranean diet component, protects against both DSS and T-cell-transfer colitis specifically through FXR activation, with protection abolished in FXR-knockout mice and independent of fermentation or SCFA production [49].

Mediterranean benefits also operate through bile acid-independent pathways: polyphenols directly inhibit NF-κB via AMPK/Nrf2, and monounsaturated fatty acids reduce inflammatory cytokines through membrane-receptor mechanisms. Bile acid signaling is one channel among several, though it is now among the better-supported mechanistic channels with direct IBD-relevant human evidence. The lipid profile improvements associated with Mediterranean diet adherence (reduced triglycerides, improved HDL metrics, and favorable LDL particle distribution) carry independent cardiovascular risk significance, particularly relevant to IBD patients who bear excess cardiovascular morbidity relative to the general population. These lipid benefits do not depend on the bile acid hypothesis, but their co-occurrence with immune improvements is the empirical observation this framework sets out to explain mechanistically.

5.2. Committed Ketogenic Configuration

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The ketogenic dietary configuration is the less certain of the two committed poles. IBD-specific human evidence is currently limited to a ten-patient case series and preclinical mouse models, none of which satisfy the data quality criteria applied throughout this paper; the mechanistic discussion below draws on this evidence for biological plausibility only, not as documentation of committed-ketosis steady-state outcomes in human IBD populations.

Sustained carbohydrate restriction below 35 g/day depletes malonyl-CoA, disinhibits CPT-1, enabling maximal hepatic β-oxidation with systemic BHB reaching 0.5–5.0 mM under nutritional ketosis as defined by the field [14,89]. The microbiome undergoes dramatic restructuring: Bifidobacterium is markedly depleted through substrate deprivation and community-level ecological effects rather than direct selective growth inhibition; pure-culture experiments showed similar BHB sensitivity across gut bacterial species [12], reducing intestinal Th17 cells in mouse models [12: Combined human (n=17, 4-week inpatient controlled feeding, 5% carbohydrate; blood BHB confirmed elevated in all participants P<0.001) and mouse mechanistic study; 4-week duration below the manuscript's ≥8-week threshold; microbiome restructuring confirmed beginning within one day of transition]. Li et al. (2024) identified specific serum taurine-conjugated bile acid species (TDCA and TUDCA) elevated under low-carbohydrate dietary conditions in mice, with correlational support from two human cohorts (n=416 observational; n=25 interventional in overweight women; ketosis not verified by blood BHB in either cohort, and carbohydrate intake in the interventional arm escalated to 36% of energy by week 12) [50: RED — no BHB verification, non-IBD obese population; admissible as mechanistic plausibility for the BSH-TGR5 pathway only]. TUDCA is a characterized TGR5 agonist and cytoprotective bile acid with demonstrated protective effects in ER stress contexts. Two recent studies directly characterize the consequences of Clostridium scindens abundance for intestinal physiology: Jalil et al. (2025) demonstrated that 7α-dehydroxylating bacteria accelerate injury-induced mucosal healing in the colon through secondary bile acid-dependent mechanisms [98], and Xiao et al. (2025) showed that C. scindens protects against cholestasis-induced liver fibrosis by activating intestinal FXR–FGF15/19 signaling [99]. BHB directly inhibits the NLRP3 inflammasome by preventing potassium efflux and ASC oligomerization, a finding replicated across laboratories independent of GPR109A, AMPK, and autophagy [51].

The favorable lipoprotein remodeling pattern under strict ketosis has been documented in humans. Westman et al. (2006), in a 24-week RCT comparing a <20g/day ketogenic program against a low-fat diet (n=119, overweight hyperlipidemic adults, compliance monitored via urinary ketones only; blood BHB not measured), demonstrated using NMR subfraction analysis statistically significant between-group reductions in large VLDL and small VLDL (both p=0.01 vs. comparator), medium LDL (−42%, p=0.02), and an increase in large LDL (+54%, p=0.004); reductions in small LDL (−78%) and large HDL (+21%) were observed within the LCKD arm but did not reach between-group significance; the LCKD arm also received fish, borage, and flaxseed oil supplementation, confounding attribution of these lipoprotein changes to ketosis per se; the study is classified TIER 2 under C-11 [86]. Favorable Treg immunology has also been separately documented: Hirschberger et al. (2021), in a prospective intervention with 44 healthy volunteers on a self-managed <30g/day protocol (counseled but not ward-controlled) with BHB verified ≥0.5 mM at days 7, 14, and 21, showed by flow cytometry that Foxp3+ Treg cells expanded significantly alongside increased IL-10 expression, with immunometabolic reprogramming toward oxidative phosphorylation confirmed by RNAseq [87]. These two findings (favorable lipoprotein subfractions and Treg expansion) have been demonstrated separately under verified committed ketosis in humans, but no study has yet measured both domains simultaneously in the same subjects under BHB-verified conditions sustained long enough to allow completed microbiome restructuring. The minimum duration for microbiome stabilization under ketogenic conditions remains unestablished in humans; Ang et al. [12] demonstrated rapid microbiome restructuring beginning within one day of diet transition in both the human and mouse components, with the 3–4-week sampling confirming structural stability rather than ongoing transition; the relevant threshold for committed-configuration signaling is not a gradual arc but a discrete shift requiring sustained maintenance. Certain early metabolic perturbations under

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KD resolved by week 12, while impaired glucose tolerance, Bifidobacterium depletion, and skeletal muscle insulin signaling changes persisted or emerged at that timepoint [73]; the full outcome-dependent temporal profile is discussed in Section 6. The lipoprotein remodeling observed under committed ketosis is most directly attributable to sustained malonyl-CoA depletion suppressing VLDL production and promoting β-oxidation; bile acid pool restructuring through microbiome changes, BHB-direct NLRP3 inhibition, and Kbhb-mediated mTOR signaling represent parallel candidate mechanisms for the immune effects, none of which has been established as the upstream coordinator. The co-directional nature of lipid and immune improvements under dietary commitment is the observation this framework sets out to explain; the mechanism remains a candidate hypothesis. This gap (verified ketosis, simultaneous multi-domain characterization, ≥12 weeks) is among the three specific design failures the Stage 1 experimental program is structured to correct.

Two IBD-relevant findings support the ketogenic configuration's biological plausibility, both from murine models. Huang et al. (2022) demonstrated that BHB levels are significantly reduced in colonic mucosa of UC and CD patients and inversely correlate with disease activity, with rectal BHB enema ameliorating DSS colitis via STAT6-dependent M2 macrophage polarization independent of gut microbiota [52: GREEN for IBD relevance of local colonic BHB deficit; does not measure dietary ketosis]. Kong et al. (2021) showed that ketogenic diet alleviates DSS colitis by reducing colonic RORγt+CD3− ILC3s and inflammatory cytokines through microbiome-dependent mechanisms confirmed by fecal microbiota transfer into germ-free mice [53: RED — mouse DSS colitis model, no BHB verification; admissible as mechanistic context for ILC3 reduction pathway]. The ILC3 reduction finding is particularly notable given ILC3s' established role in sustaining colonic Th17 programs in IBD and warrants prospective human investigation.

Clinical evidence in IBD patients currently rests at case-series level. Norwitz and Soto-Mota (2024) reported 10 IBD patients (6 UC, 4 CD) on carnivore-ketogenic diets achieving universal clinical improvement, with most discontinuing medications [54: AMBER — signal only; no BHB documentation in C-11 format; the only IBD-specific human clinical signal for the ketogenic pole]. These data are insufficient for clinical inference but provide signal warranting prospective investigation.

Critical Caveats on Ketogenic Lipid and Atherosclerosis Data

Most published studies reporting lipid outcomes under 'ketogenic' or 'low-carbohydrate' diets did not verify sustained ketosis. Bravata et al. reviewed 107 studies captured by low-carbohydrate search terms with carbohydrate content ranging from 0 to 901 g/day; of these, only 38 diets prescribed ≤60 g/day [13]. A 2025 narrative review of BHB testing in ketogenic metabolic therapies reports daily compliance ranging from 63% to 89% across psychiatric case reports and small pilot studies [14]; these figures derive from studies of one to approximately twenty participants and cannot be generalized to clinical trial populations. The KETO trial (Budoff et al., n=80 per arm) found no coronary plaque difference between Lean Mass Hyper-Responder individuals (mean LDL-C 272 mg/dL) and matched controls in cross-sectional comparison within this self-selected phenotype [15]. The longitudinal follow-up study (Soto-Mota et al., 2025) was subsequently retracted by JACC Advances due to methodological concerns; no reliable longitudinal plaque progression data from this cohort are currently available [16].

The lipid and atherosclerosis claims in this paper are therefore stated with appropriate uncertainty: whether strict ketosis consistently produces favorable cardiovascular outcomes across populations remains an open and actively contested question. Studies that appear to show ketogenic diets producing adverse or null inflammatory outcomes typically share one or more characteristics: the dietary intervention was not verified through blood BHB measurement; the carbohydrate intake studied exceeded the threshold required to establish sustained β-oxidation; or inflammatory markers were measured during the early transition period rather than at metabolic steady state. The Virta Health cohort demonstrated the metabolic and lipid benefits achievable under a nutritional ketosis

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intervention [10,11], but mean laboratory BHB at one year was 0.30 mmol/L across the full cohort [75], below the ≥0.5 mM committed-ketosis threshold, indicating that a substantial proportion of participants did not achieve committed ketosis as defined here, and that the reported lipid and immune outcomes should be interpreted accordingly [AMBER].

The adverse lipid signal in lean individuals merits specific attention. Burén et al. (2021), in a randomized controlled feeding trial providing all food to healthy normal-weight women (n=17, mean BMI 21.9 kg/m²) on a verified ketogenic diet (<25g/day carbohydrates, BHB confirmed ≥0.5 mM by blood measurement in every participant at study endpoint (day 29); daily compliance monitored throughout by urinary ketones), found that both large buoyant LDL (+31.56 mg/dL, $p<0.001$) and small dense LDL (+4.51 mg/dL, $p<0.01$) increased significantly, alongside a rise in ApoB (+0.50 g/L, $p<0.001$) [88]. The predominant increase was in large buoyant particles, consistent with the LMHR phenotype, but the small dense LDL fraction also increased significantly, and total atherogenic particle burden rose. This finding, in the population most likely to achieve verified ketosis under controlled conditions, indicates that the lipid response to committed ketosis in lean individuals cannot be characterized as uniformly favorable by conventional metrics, and that NMR subfraction analysis is required to distinguish between qualitatively different lipid responses across individuals.

The timing issue is particularly important in the IBD context. Ang et al. [12] established that microbiome restructuring is a gradual ecological process requiring weeks to stabilize. A recent report documented increased small intestinal permeability and elevated serum LPS in obese subjects following eight weeks of very low calorie ketogenic diet [17]; the study was conducted without blood BHB verification. This finding is consistent with the transition-state inflammatory signal this framework predicts prior to microbiome stabilization, rather than with the post-stabilization committed configuration the hypothesis describes. Whether eight weeks falls within or beyond the microbiome restructuring window in the IBD colon, where baseline dysbiosis may alter the stabilization trajectory, cannot be determined from currently available data. This observation is neither confirmatory nor contradictory with respect to the framework's IBD-relevant predictions; it confirms the need for longitudinal studies with verified ketosis and sufficient follow-up.

The metabolic lipid benefits of verified sustained ketosis (decreased small LDL particle number, reduced large VLDL particle concentration, and sustained triglyceride and HDL improvements) represent clinically meaningful outcomes in metabolically appropriate populations, independent of any immunological claim. That these improvements occur alongside immune remodeling in the committed-ketosis configuration is the empirical co-occurrence motivating the shared-mechanism hypothesis; each dimension retains clinical significance whether or not they prove mechanistically linked, and whether or not the lipid response is uniformly favorable across lean versus metabolically dysregulated individuals.

The widely cited claim that BHB inhibits class I HDACs [55] has been directly challenged [56], and subsequent research has converged on lysine β-hydroxybutyrylation (Kbhb) as the operative BHB-specific epigenetic modification, with Qin et al. (2024) demonstrating that ketogenic diet reshapes metabolism primarily through Kbhb (including ALDOB K108bhb inhibiting mTOR signaling) rather than classical HDAC inhibition [57,58]. The Kbhb evidence reinforces rather than complicates the dietary commitment argument: metabolic reshaping through whole-diet microbiome and bile acid remodeling, rather than circulating BHB concentration per se, is the operative mechanism the framework proposes.

6. The Intermediate Zone: A Question, not a Claim

The intermediate zone hypothesis did not originate in the literature. It originated in a clinical anomaly. Published low-carbohydrate diet trials frequently reported adverse lipid outcomes (elevated LDL, worsened atherogenic indices) while practitioners following strictly enforced very-low-carbohydrate protocols reported the opposite. O'Neill, Westman, and Bernstein (2003) documented this divergence directly in a strictly enforced ≤30g carbohydrate protocol, with favorable shifts across all atherogenic lipid indices [77]; generalizability to non-diabetic and IBD-specific

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populations remains to be established. Outside that strictly enforced threshold, the low-carbohydrate literature shows LDL increases particularly pronounced in lean individuals [78], a finding that extends to the atherogenic small dense LDL fraction under verified ketosis in lean women, as Burén et al. (2021) demonstrated [88]. The most parsimonious explanation for the divergence between strictly enforced and loosely enforced protocols is not methodological artifact but metabolic state heterogeneity: most published "ketogenic" trials studied carbohydrate-restricted but non-ketotic subjects, individuals occupying what the present analysis terms the intermediate zone, rather than the strict carbohydrate restriction under which the divergent lipid outcomes were observed.

The adverse lipid pattern in the intermediate zone is not a scattered finding. Among studies reviewed by Bravata et al. [13], the 69 studies prescribing more than 60 g/day of carbohydrate (well above the ketogenic threshold) constitute the majority of the low-carbohydrate literature and contain the bulk of the reports of elevated LDL and worsened atherogenic indices. The Virta Health cohort, with mean BHB of 0.30 mmol/L at one year [75], falls below the committed-ketosis threshold; yet its outcomes are routinely cited as evidence of ketogenic diet effects. The meta-analytic finding that LDL increases under low-carbohydrate diets are pronounced specifically in lean individuals [78] is consistent with the intermediate-zone interpretation: lean individuals are less likely to achieve sustained ketosis under a given carbohydrate gram target than individuals with greater metabolic insulin resistance, making them over-represented in the intermediate zone within any nominally ketogenic trial. The adverse lipid data in the low-carbohydrate literature are consistent with evidence of intermediate-zone signaling incoherence rather than committed-ketosis physiology. Alternative explanations including differences in dietary protein, omega-6:omega-3 ratio, total fiber, and caloric restriction between strictly- and loosely-enforced protocols cannot be excluded without controlled comparison.

The mechanistic basis for intermediate-zone instability is not merely the absence of ketosis. Periodic carbohydrate intake generates rapid malonyl-CoA elevation (documented at approximately 2.7-fold within hours of hyperglycemia with hyperinsulinemia [59]) that intermittently inhibits CPT-1, preventing the establishment of sustained β-oxidation. Each carbohydrate exposure resets this inhibition, so the metabolic state oscillates between partial fat oxidation and glucose utilization without stabilizing in either committed configuration. The microbiome responds to this substrate oscillation by occupying a transitional community state, neither the Bifidobacterium-depleted ketogenic configuration nor the diverse saccharolytic Mediterranean configuration, producing a bile acid signaling environment that is neither coherently pro-tolerogenic nor coherently immunostimulatory. The net effect, under this framework, is predicted to default toward immunostimulation through the same mechanisms that generate coherent tolerance under committed patterns, operating in reverse: incomplete secondary bile acid generation, partial rather than sustained NLRP3 suppression, and oscillating rather than stable FXR and TGR5 receptor engagement. The mechanistic basis for this directionality is NLRP3 oscillation: intermittent BHB-mediated inhibition followed by rebound assembly during uninhibited periods generates a net-inflammatory signal not present under either committed configuration. Whether this metabolic instability constitutes a reproducible and distinct signaling configuration, rather than simply the expected variance of incomplete dietary commitment, is the central empirical question Section 9's Stage 1 design addresses.

The duration of dietary commitment matters independently of carbohydrate gram count. Hengist et al. (2024, Cell Reports Medicine) demonstrated in an RCT that certain early metabolic perturbations under KD (including fasting glucose, apoB, and CRP) resolved by week 12 [73]; however, impaired glucose tolerance, Bifidobacterium depletion, and skeletal muscle insulin signaling changes persisted or emerged at that timepoint in the same trial, indicating that temporal resolution of KD-associated metabolic effects is outcome-dependent. This finding supports the duration threshold argument directly: early metabolic perturbations during the microbiome transition period are predicted by the framework, and their partial normalization under sustained commitment is consistent with the committed-configuration signal emerging only after microbiome

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stabilization. Ang et al. [12] established in mouse models that microbiome restructuring requires weeks to stabilize; the Hengist et al. human RCT data provide the first direct evidence that a similar transition-to-stabilization arc occurs in humans, though the IBD-specific timeline remains uncharacterized.

Two levels of claim are at issue here, and they should not be conflated. The observation: intermediate carbohydrate restriction has not been shown to produce the lipid or inflammatory improvements seen with committed patterns, and most 'ketogenic' trials reporting adverse lipid effects likely studied this zone rather than verified ketosis. The mechanistic hypothesis: bile acid signaling incoherence contributes to these suboptimal outcomes and, specifically, to insufficient restoration of the immunomodulatory trace bile acid species depleted in IBD. Both levels of claim require prospective testing; neither is established.

Before proceeding, the framework's three qualitative groups warrant explicit definition, because the gram-count boundaries used throughout the literature are imprecise proxies for distinct physiological states.

The committed ketogenic group is defined primarily by a metabolic criterion: sustained blood BHB ≥0.5 mM [14,89]. This threshold is a clinical convention marking reliable entry into nutritional ketosis as a metabolic state; it is not a validated immunological switch. Published in vitro data for BHB-dependent immune mechanisms (NLRP3 inhibition, Kbhb-mediated epigenetic Treg programming) largely use concentrations of 1–10 mM; the immunologically relevant BHB range in committed ketosis is therefore likely the upper portion of the 0.5–5.0 mM physiological range rather than the threshold itself. Carbohydrate gram targets are population-level proxies for this metabolic state rather than the state itself. The committed Mediterranean group is defined by documented adherence to the dietary pattern associated with the PREDIMED and CORDIOPREV outcomes: high fiber, predominantly plant-based, rich in MUFAs, limited in ultra-processed foods. The intermediate zone is defined by the absence of verified ketosis in an individual nominally following a low-carbohydrate approach. The gram-count boundaries cited here (50–150 g/day as a rough intermediate range) are practical reference points for study design, not biologically precise thresholds. This is the most speculative element of the present framework: no human study has yet performed bile acid metabolomics across a carbohydrate restriction gradient in IBD patients or in healthy individuals characterized for Th17/Treg ratio.

Population mortality data across carbohydrate intake gradients do not characterize a mechanistically distinct intermediate zone; whether one exists remains an empirical question the Stage 1 design addresses directly [60,61]. The intermediate zone conflates two biologically distinct sub-states that current measurement practices do not distinguish. The first is consistent moderate restriction: stable carbohydrate intake at 20–45% of energy, maintained such that blood BHB remains below 0.5 mM throughout. The second is oscillating restriction: nominally low-carbohydrate intake in which carbohydrate varies across days, causing BHB to fluctuate above and below the 0.5 mM ketotic threshold. These sub-states have divergent predicted effects. Consistent moderate restriction may better preserve SCFA-producing microbiota. It may also avoid the impaired glucose tolerance documented in keto-adapted subjects upon carbohydrate re-exposure [92,93]. This pattern has been demonstrated in rodent models and cross-sectional human data but not in longitudinal dietary cycling protocols. Oscillating restriction may intermittently engage BHB-dependent immune mechanisms, including NLRP3 suppression and Treg differentiation through epigenetic programming [94]. At the same time, repeated dietary transitions subject the microbiome to reconfiguration events whose net consequences for mucosal immune status are uncertain. Whether either sub-state is superior across the metabolic and immune outcomes relevant to IBD, or whether any advantage is outcome-dependent, cannot be determined from available data; no published study has directly compared these sub-states against each other or against committed ketosis in a single trial. The Intermediate Oscillation Index is designed to capture this variability within the intermediate group; sub-stratification by dietary pattern stability is a planned secondary analysis. One asymmetry in the BHB verification standard applied here warrants explicit acknowledgment:

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Westman et al. (2006) [86] is cited in Section 5.2 as the only available NMR subfraction dataset under a strictly enforced carbohydrate protocol, despite verification resting on urinary ketones alone rather than blood BHB measurement; this is the same verification gap that places other studies in the intermediate zone. That citation is retained not as committed-ketosis evidence but as the sole available source for NMR subfraction analysis under strict carbohydrate restriction, with its limitation disclosed; it is classified as TIER 2 accordingly. (Figure 2)

7. Oxysterols, LXR, and the Cholesterol–Bile Acid–Immune Interface

Bile acids and oxysterols share a common biosynthetic origin: both derive from hepatic cholesterol, with bile acid synthesis proceeding via CYP7A1 and CYP8B1 under FXR-mediated feedback control, and oxysterol generation proceeding via CYP27A1 and CYP7B1 under LXR-mediated regulation. This shared substrate creates a coupled regulatory circuit in which dietary patterns that shift hepatic cholesterol flux alter both the bile acid pool and the oxysterol landscape simultaneously, engaging FXR, TGR5, LXRα, and LXRβ as co-equal nodes in the same network rather than as parallel independent systems. The oxysterol-LXR axis described in this section is therefore not a supplementary layer intersecting the bile acid framework; it is part of the same coupled network, sharing substrates, regulatory nodes, and immune effectors, whose integrative architecture is considered in the Discussion.

Parigi et al. (2021, Mucosal Immunology) provided the most mechanistically relevant demonstration in gut-specific context, showing that LXRα deficiency increased mesenteric Th17 cells specifically, while LXRβ deficiency increased RORγt+ Tregs specifically, through signaling in CD11c+ myeloid cells [62]. These are non-overlapping isoform-specific functions with distinct downstream cell types. Jacobse et al. (2023) extended this by demonstrating that IL-23R signaling downregulates LXR target genes in colonic Tregs, impairing their stability and function; an LXR inverse agonist decreased colonic Treg frequency, and human scRNA-seq of Crohn's ileal lamina propria confirming IL-23R expression on Tregs [63]. These findings place LXR as a shared regulatory node connecting sterol metabolism, cytokine signaling, and mucosal Treg maintenance in IBD-relevant tissue.

The specific oxysterol 27-hydroxycholesterol (27-OHC), produced from cholesterol by CYP27A1 in macrophages and hepatocytes, upregulates RORγt and promotes Th17 differentiation while suppressing Tregs [64,65] (data from neurological and cognitive mouse models; direct demonstration in gut-relevant colonic immune cell populations has not been established); this effect is reversed by the RORγt inhibitor SR1001, confirming the RORγt-dependent mechanism. This places 27-OHC in functional antagonism with 3-oxoLCA and isoLCA at the level of RORγt-mediated gene regulation, each acting through RORγt-dependent pathways to produce opposing immunological outcomes; whether they compete for the same binding site or act through distinct conformational mechanisms has not been established. In tumor-associated macrophages, lysosomal 25-hydroxycholesterol (25-HC) competes with cholesterol for GPR155 binding, inhibiting mTORC1 and activating AMPKα to drive STAT6-dependent immunosuppressive reprogramming [66]; whether this pathway operates in colonic macrophages under dietary conditions relevant to IBD has not been studied. The structurally related oxysterol 7α,25-dihydroxycholesterol signals through GPR183/EBI2 on innate lymphoid cells, directing their positioning in colonic lymphoid tissue [67]. These distinct oxysterol species thus act through distinct receptors on distinct cell types, providing complementary regulatory layers relevant to intestinal homeostasis. GPR183-mediated ILC3 positioning in colonic lymphoid tissue has been demonstrated as a pathogenic driver in experimental colitis [67], and 7α,25-dihydroxycholesterol administration significantly decreased immune cell counts in mesenteric lymph nodes in a sex-specific manner during DSS colitis [68].

These oxysterol-LXR-immune interactions are speculative in the specific context of dietary pattern modulation; no published study has directly measured oxysterol profiles under committed KD or Mediterranean diet conditions in IBD patients. The framework proposes that dietary patterns producing coherent bile acid environments also remodel the cholesterol intermediate and oxysterol landscape through altered CYP7A1 flux reducing cholesterol availability for oxysterol synthesis, and

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through FXR-LXR crosstalk at shared gene regulatory networks. Committed KD, by depleting malonyl-CoA and shifting hepatic lipid flux toward β-oxidation, would be expected to reduce de novo cholesterol synthesis and potentially alter 27-OHC production in macrophages. This prediction is testable and should be incorporated into the Stage 1 metabolomic panel. The oxysterol-LXR circuit described here is therefore not a parallel or supplementary framework but a proposed component of the same coupled network, sharing substrates, regulatory nodes, and immune effectors with the bile acid axis, whose integrative architecture is considered in the Discussion. Whether committed ketogenic diet reduces hepatic cholesterol synthesis sufficiently to alter macrophage 27-OHC production at immunologically relevant magnitudes remains a speculative prediction requiring direct oxysterol profiling in human subjects.

Mendelian randomization provides indirect support for this cholesterol–immune node: HMGCR-mediated LDL-C lowering did not increase IBD risk, while PCSK9-mediated LDL-C lowering paradoxically did [69], suggesting that the cholesterol synthesis pathway modulates IBD-relevant inflammatory signals through pleiotropic mechanisms beyond simple lipid reduction, consistent with the framework's predicted FXR–LXR crosstalk at shared gene regulatory networks. These receptor-level findings point toward a broader coordination problem, one the following section attempts to address at the anatomical level.

8. Systems-Level Coordination: The Hepatic-Vagal-Colonic Arc

Distributed coordination between metabolism and immunity operates across multiple anatomical layers. At the humoral level, GLP-1 secreted by L-cells in response to bile acids (TGR5) and SCFAs (GPR43) suppresses hepatic lipogenesis and modulates innate immune tone; GLP-1 analogue therapy reduces inflammatory cytokine production in immune cells [72], providing biological plausibility for bile acid-driven GLP-1 secretion as a candidate link to systemic immune regulation. This humoral layer complements the receptor-mediated signaling through FXR, TGR5, S1PR2, and RORγt described in Section 2, together constituting the two best-supported coordination mechanisms by which dietary commitment generates coherent metabolic and immune outputs across tissue compartments.

A neural coordination layer has also been proposed. Teratani et al. identified a liver–brain–gut arc in which hepatic vagal sensory afferents detect portal blood metabolites and relay through the nucleus tractus solitarius to generate efferent vagal output maintaining the colonic Treg cell niche [70]; bile acids are plausible portal metabolite candidates given their concentration dynamics, but this attribution remains an inference. This finding has not been independently replicated outside the originating Kanai laboratory; technically demanding lateralized microsurgical procedures may explain the absence of replication attempts, which is distinct from failed replication. The Teratani arc is a plausible but unconfirmed coordination mechanism; its structural weight in this framework is proportionally lower than the receptor-mediated and humoral layers, which rest on replicated evidence across multiple laboratories and model systems.

Experimental Program

9. A staged Experimental Program

Prior studies of ketogenic diet effects on lipid and immune outcomes share three specific design failures that have prevented interpretable results in IBD-relevant populations. First, the majority did not verify sustained ketosis through blood BHB measurement, leaving committed-ketosis physiology indistinguishable from intermediate-zone biology in their outcomes [13]. Second, most measured outcomes during the microbiome transition period rather than at metabolic steady state. Ang et al. [12] demonstrated rapid microbiome restructuring beginning within one day of KD transition, with 3–4-week sampling confirming structural stability; the outcome-dependent temporal profile shown in a human RCT [73] together set a minimum duration threshold that most published trials did not reach. Third, no study has simultaneously characterized dietary metabolic state, the bile acid and

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SCFA metabolome, and Th17/Treg balance in the same subjects, the three measurement domains whose co-variation is necessary to test whether the co-directional improvements observed under committed dietary patterns share a common mechanistic basis. A planned secondary analysis will stratify enrolled committed-ketosis participants by BHB quartile within the ≥0.5 mM range to test whether bile acid and immune endpoints scale with BHB concentration within the committed group; this addresses the acknowledged gap between the 0.5 mM enrollment floor and the 1–10 mM range at which BHB-dependent immune mechanisms have been demonstrated in vitro. The staged program described here is designed to correct all three failures in sequence.

Rather than proposing a single definitive trial, the present paper outlines a staged program designed to be practical, sequentially informative, and feasible at academic core facility rates. The program tests two related but separable questions: first, whether committed dietary patterns produce systematically different co-directional lipid and immune outcomes than intermediate carbohydrate restriction (the empirical question, which has value independent of the mechanistic hypothesis); and second, whether bile acid profile changes mediate those differences if they are confirmed (the mechanistic question). A negative finding on the mechanistic question would not nullify a positive finding on the empirical question. The study design incorporates an IBD-enriched arm specifically to test whether the bile acid–Treg axis is disrupted in a manner consistent with the framework's predictions in disease-relevant tissue. (Figure 3)

Stage 1: Cross-sectional bile acid profiling across dietary patterns with an IBD cohort. Recruit four cohorts of 50–75 individuals each: persons on verified strict ketogenic diet (≥12 weeks, documented BHB ≥0.5 mM [14,89]); persons on documented Mediterranean diet (≥12 weeks, MEDAS score ≥9 with ASA24 24-hour dietary recall); persons consuming intermediate carbohydrate restriction (50–150 g/day) without verified ketosis; and patients with quiescent IBD (Mayo score ≤1 for UC and Harvey-Bradshaw ≤4 for CD, with fecal calprotectin <150 μg/g confirming mucosal quiescence at enrollment, on stable maintenance therapy) consuming either committed or intermediate dietary patterns. The IBD arm will be stratified by maintenance therapy class at enrollment: biologic therapy (anti-IL-23 agents including ustekinumab, risankizumab, and mirikizumab; anti-TNF agents), aminosalicylate monotherapy, and no maintenance therapy. Sensitivity analyses will examine Th17/Treg and bile acid outcomes within each stratum separately, to distinguish pharmacological suppression of the Th17 axis from dietary effects. Perform targeted bile acid metabolomics (including 3-oxoLCA, isoalloLCA, isoLCA, isoDCA, BA-MCY conjugates, conjugated and unconjugated primary and secondary species), oxysterol profiling (27-OHC, 25-HC, 7α,25-diHC), NMR lipoprotein subfraction analysis, and targeted immune panel (Th17/Treg ratio by flow cytometry, hsCRP, IL-6, fecal calprotectin for IBD arm). Statin use (any statin at stable dose) will be recorded for all participants and used as a stratification variable in sensitivity analyses of the oxysterol panel findings, given the pleiotropic effects of HMG-CoA reductase inhibition on S1PR2/SphK1 signaling and LXR-mediated Treg stability in IBD [36–38,62]. Within the intermediate carbohydrate restriction cohort, a candidate Intermediate Oscillation Index (IOI) is proposed to sub-stratify participants by degree of metabolic instability. IOI combines BHB response to a standardized 50g oral carbohydrate challenge (fasting BHB at baseline, re-measured at 2 hours post-challenge) with intra-individual carbohydrate intake variance across a 7-day ASA24 dietary recall period. The index captures the degree to which BHB fluctuates across the 0.5 mM threshold as dietary carbohydrate varies. IOI is a novel construct with no prior validation; the operational formula and full challenge protocol are provided in Supplementary File 1. IOI validation is a Stage 1 secondary objective, not a Stage 1 assumption: a feasibility assessment in n=10–15 intermediate-zone participants is proposed before committing IOI to the primary analytical plan. If feasible within Stage 1, IOI values will be tested for correlation with HMCRI and Th17/Treg ratio as an exploratory probe of whether metabolic oscillation within the intermediate zone tracks with the signaling disruption the framework predicts. The primary analytical aim is to determine whether bile acid profiles differ between dietary groups and correlate with simultaneous lipid and immune outcomes. The IBD arm will test whether patients with quiescent IBD on committed dietary patterns demonstrate restoration of immunomodulatory

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trace bile acid species relative to those on intermediate patterns. The IBD arm will be enriched with a PSC sub-cohort (n=30–40, contingent on hepatology center collaboration), stratified by VNN1 genotype and BA-MCY conjugate levels (subject to platform feasibility). VNN1 encodes pantetheinase, the enzyme responsible for BA-MCY conjugate production; genotypic variation in VNN1 expression has been documented in IBD and colitis contexts and may directly modulate the host counter-regulatory bile acid pool [21,100,101]. Specific VNN1 variants for genotyping will be selected based on platform feasibility and published functional annotation at the time of Stage 1 protocol development, and will be pre-registered as a secondary analytical objective before enrollment begins. Genotype-stratified analysis of immunomodulatory trace bile acid concentrations and Th17/Treg outcomes will provide a direct test of the inference chain identified in Section 3 as the framework's most critical unmeasured gap: whether depletion of bile acid-transforming bacteria in PSC produces quantifiably reduced immunomodulatory trace species that correlate with Th17/Treg imbalance independently of dietary pattern. Estimated cost: $200,000–$400,000 at academic core facility rates, dominated by targeted LC-MS/MS bile acid metabolomics, NMR lipoprotein subfraction analysis, and multi-parameter flow cytometry across four cohorts of 50–75 participants. Recruitment of quiescent IBD patients already committed to a verified ketogenic diet for ≥12 weeks represents a practical challenge unlikely to be resolved within a single gastroenterology practice; multi-center enrollment across academic IBD centers and patient advocacy network partnerships are the anticipated recruitment solutions.

Stage 2: Bile acid mediation analysis in existing trial biobanks. The PREDIMED, DIRECT-PLUS, Virta Health, and, critically, the Haskey UC Mediterranean diet RCT (NCT03053713) cohorts have stored biological samples. Retrospective bile acid metabolomics on stored samples, combined with existing lipid and inflammatory marker data, could test whether bile acid profile changes mediate observed dietary effects through structural equation modeling. Estimated cost: $100,000–$300,000 per biobank accessed.

Stage 3: Prospective three-arm dietary intervention with multi-omic profiling. If Stages 1–2 confirm bile acid–mediated correlations, a prospective RCT with three arms (strict KD with daily BHB verification, Mediterranean with dietary recall, intermediate restriction at 75–100 g carbohydrate) and serial sampling at weeks 0, 2, 4, 8, and 12 would provide definitive causal evidence. Required sample size depends on effect sizes observed in Stages 1–2. The design must include a planned mediation arm testing BHB-direct vs. bile acid–mediated immune effects, because BHB-direct NLRP3 inhibition (Youm et al. [51]) and Kbhb-mediated mTOR signaling (Qin et al. [58]) represent currently more parsimonious explanations for some ketosis-associated anti-inflammatory effects; Stage 3 must formally distinguish these mechanisms from the bile acid hypothesis. Estimated cost: $1–5 million depending on design.

The staged experimental program measures BA-MCY conjugates and the four principal immunomodulatory secondary bile acid species as separate analytes. The HMCRI is proposed to capture the net balance between the host counter-regulatory pool (BA-MCY conjugates, acting as FXR antagonists) and the microbially generated immunomodulatory pool. The underlying hypothesis is that this balance determines net receptor engagement and Th17/Treg outcomes more directly than absolute species concentrations. The denominator species operate through distinct receptor pathways: 3-oxoLCA acts as a RORγt inverse agonist in T cells [18] and as an FXR agonist in intestinal epithelial cells [91]; isoDCA acts as a functional FXR antagonist on dendritic cells [19] but as an FXR agonist in heterologous FXR reporter systems [31]; isoalloLCA induces Foxp3 expression through mitochondrial ROS and NR4A1 signaling, independently of FXR [18,30]; isoLCA suppresses Th17 differentiation via RORγt binding with no confirmed direct FXR engagement [4]. HMCRI is therefore a mixed-mechanism immunomodulatory index, not a pharmacologically uniform FXR balance ratio; its biological rationale is empirical: the four species are those specifically depleted in IBD cohorts [4], selected on the basis of observed depletion rather than shared receptor pharmacology. The denominator treats these species as equivalent units of immunomodulatory contribution, setting aside their mechanistic heterogeneity to construct a tractable index; Stage 1 data will allow revision

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toward a species-weighted formulation informed by empirical potency estimates, with individual species concentrations reported alongside HMCRI to permit interpretation independent of the aggregate ratio. HMCRI is defined as:

$$\text{HMCRI} = \text{BA-MCY conjugates} / (\text{3-oxoLCA} + \text{isoalloLCA} + \text{isoLCA} + \text{isoDCA})$$

The numerator is the host counter-regulatory antagonist pool. Won et al. (2025) identified VNN1 as one mechanism by which the host generates BA-MCY conjugates that act as potent FXR antagonists [21]; VNN1 may not be the sole source, and the full enzymatic landscape of BA-MCY production remains under active characterization. The denominator is the microbially generated immunomodulatory pool. These four species are produced through two enzymatically distinct pathways: 3-oxoLCA and isoalloLCA arise through Coriobacteriaceae-mediated 3α/3β-hydroxysteroid dehydrogenase activity, while isoLCA and isoDCA require Clostridium scindens and related taxa for 7α-dehydroxylation and subsequent epimerization [4,18,19].

A low HMCRI indicates that microbially generated immunomodulatory species predominate, the predicted configuration under committed dietary patterns at either pole, where intact Coriobacteriaceae and Clostridium scindens populations sustain secondary bile acid biosynthesis. A high HMCRI can arise through two distinct mechanisms that the ratio alone cannot separate: depletion of the microbial denominator species, or elevation of host BA-MCY production, or both simultaneously. In IBD and PSC patients with documented Coriobacteriaceae depletion [4,5,7], a high HMCRI driven primarily by denominator depletion is the predicted pattern regardless of dietary commitment, a finding that would indicate host counter-regulatory activity operates as a disease-state phenomenon separable from the dietary signal, requiring explicit integration into the framework. In the intermediate carbohydrate restriction zone, incomplete microbiome restructuring is the primary predicted driver. Distinguishing these two mechanisms requires examining numerator and denominator components separately alongside the ratio, which the Stage 1 measurement panel is designed to permit.

All five components are quantifiable by targeted LC-MS/MS. BA-MCY fecal quantification protocols have not been independently validated in humans; serum-based measurement is confirmed feasible per Won et al. [21]. Matrix selection for BA-MCY analytes will depend on platform validation at participating core facilities prior to Stage 1. If serum BA-MCY is used as the numerator while denominator species are measured from fecal samples, the ratio spans two biological compartments: serum BA-MCY reflects systemic FXR antagonist load, while fecal denominator species reflect luminal immunomodulatory availability. This limits HMCRI's interpretation as a luminal signaling coherence index. Numerator and denominator will be reported separately to permit interpretation independent of the cross-compartment ratio. Human population reference ranges for HMCRI do not exist; establishing them across the four Stage 1 cohorts is a specific analytical objective. HMCRI is a candidate measurement construct requiring empirical validation and not a validated clinical tool. Its falsification criterion: if HMCRI does not differ between committed and intermediate dietary groups despite differing lipid and immune outcomes, bile acid signaling coherence is non-operative as the proposed intermediate. That interpretation requires ruling out assay sensitivity limitations and population heterogeneity as alternative explanations before the mechanistic conclusion is drawn. HMCRI is introduced in the Stage 1 measurement protocol in Section 9 and the candidate index sentence in Section 11.

10. Testable Predictions

The framework generates specific predictions that distinguish it from the null hypothesis of independent metabolic and immune effects. Prediction 1: committed dietary patterns at either pole will differ from intermediate carbohydrate restriction in co-directional improvements in both lipoprotein subfractions and Th17/Treg balance. The Stage 1 design tests bile acid profile as the primary proposed intermediate. This choice is specific: bile acid metabolomics is the only candidate mechanism measurable in a cross-sectional design that links dietary metabolic state to both output

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domains through a single analytical platform. Structural equation modeling will test whether immunomodulatory bile acid concentrations mediate the relationship between dietary group and both outputs, using pooled data across all four cohorts with informative priors derived from Hang 2019 [18], Campbell 2020 [19], and Paik 2022 [4]. Per-arm SEM is not powered at Stage 1 enrollment targets; per-arm mediation analysis is deferred to Stage 3, where sample sizes will be set based on Stage 1 effect estimates. If committed groups differ in outputs but not in bile acid profiles, bile acid signaling is non-operative; BHB-direct NLRP3 inhibition and Kbhb-mediated mTOR signaling become the primary candidates for Stage 3 prospective testing.

Prediction 2: bile acid mediation will operate through multiple receptor-specific pathways (3-oxoLCA/RORγt, isoDCA/FXR antagonism, TGR5/GLP-1), not through a single FXR-SHP pathway. Prediction 3: host BA-MCY conjugate levels (Won et al. 2025 [21]) will differ between dietary groups and contribute to the host-microbe immunomodulatory balance. Prediction 4: during dietary transition (weeks 1–4), a transient inflammatory elevation will coincide with microbiome restructuring and will be most pronounced in the intermediate arm, where restructuring is predicted to be incomplete. The anti-inflammatory effect of the committed ketogenic arm is predicted to require whole-diet microbiome remodeling rather than circulating BHB alone, consistent with evidence that exogenous BHB supplementation does not replicate the ketogenic diet's anti-colitis effects [85]. Prediction 5: PSC patients and IBD patients with documented microbiome depletion of Gordonibacter and Eggerthella (per Paik et al. [4]) will have lower concentrations of 3-oxoLCA, isoLCA, and isoalloLCA than patients with intact Coriobacteriaceae populations; the PSC microbiome depletion of Eubacterium spp. and Ruminococcus obeum documented by Kummen et al. [5] represents the upstream loss of enzymatic capacity predicted to produce this deficit. Th17/Treg ratios should inversely correlate with trace bile acid levels across both disease contexts. Prediction 6: oxysterol profiles, specifically 27-OHC, will differ between dietary groups in a direction consistent with reduced hepatic cholesterol intermediate availability under committed KD, and will correlate inversely with immunomodulatory secondary bile acid concentrations. Prediction 6 is designated exploratory, consistent with the H4 designation in Table 3; supporting evidence derives from neurological and cognitive mouse models and direct demonstration in gut-relevant colonic immune cell populations has not been established.

Competing mechanistic hypotheses make distinguishable predictions across the Stage 1 outcome domains (Table 3).

Table 3. Discriminative predictions of competing mechanistic hypotheses across Stage 1 outcome domains.

Outcome Domain	H1: Bile Acid Signaling	H2: BHB-Direct NLRP3 Inhibition	H3: Kbhb-mTOR / Epigenetic	H4: Oxysterol-LXR (Exploratory)	Decision Rule
Immunomodulatory bile acid concentrations (3-oxoLCA, isoalloLCA, isoLCA, isoDCA)	Predicted higher in committed vs. intermediate groups; HMCRI predicted lower. Mechanistic rationale: these species suppress Th17 via RORγt binding and expand Tregs via	No specific prediction; BHB-direct NLRP3 inhibition operates through K^+ efflux blockade [51]; relationship to bile acid pool composition not tested.	No specific prediction; Kbhb operates through histone modification [57] and ALDOB K108bhb-mediated mTOR inhibition [58]; relationship to bile acid concentrations untested.	Predicted to correlate inversely with 27-OHC via shared RORγt competition; mechanistic rationale from mouse models [62,64]; gut-specific demonstration not established. Exploratory.	Bayesian SEM + LOO-CV

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Outcome Domain	H1: Bile Acid Signaling	H2: BHB-Direct NLRP3 Inhibition	H3: Kbhb-mTOR / Epigenetic	H4: Oxysterol-LXR (Exploratory)	Decision Rule
	mitochondrial ROS [18,19]; their depletion is documented in IBD cohorts [4], not in dietary adherence groups. BA-MCY FXR antagonist biology established in mouse and limited human serum data [21]; dietary-group predictions are untested.				
Th17/Treg balance (flow cytometry)	Predicted improved in committed vs. intermediate; immunomodulatory bile acid concentrations predicted to mediate this improvement in SEM [18,19]. HMCRI predicted to track direction of effect [21]. Discriminating feature: H1 predicts bile acid mediation of both lipid and immune outcomes simultaneously; neither H2 nor H4 predicts this co-mediation. All dietary-group predictions are untested.	BHB inhibits NLRP3 assembly via K^+ efflux blockade in macrophages and human monocytes [51]; predicts Th17/Treg benefit in committed KD arm specifically, not Mediterranean arm (BHB concentration-dependent). Bile acid independence inferred, not directly tested.	Not directly testable in Stage 1; BHB concentration serves as indirect proxy for Kbhb substrate availability only [57,58]. Formal test planned for Stage 3 (sorted Treg Kbhb ChIP-seq).	LXR deficiency increases mesenteric Th17 in mouse models [62]; IL-23R suppresses LXR target genes in colonic Tregs [63]. 27-OHC promotes Th17 via RORγt in neurological models [64,65]; gut demonstration not established. Exploratory.	Bayesian SEM + LOO-CV

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Outcome Domain	H1: Bile Acid Signaling	H2: BHB-Direct NLRP3 Inhibition	H3: Kbhb-mTOR / Epigenetic	H4: Oxysterol-LXR (Exploratory)	Decision Rule
Lipoprotein subfractions (NMR)	Favorable subfraction profile predicted in committed groups. FXR activation suppresses VLDL-TG secretion via SHP-SREBP-1c repression [22,23] and is a candidate partial mediator; malonyl-CoA/CPT-1 disinhibition is the more direct explanation for KD remodeling and is not a bile acid mechanism. H1 predicts both outcomes co-mediated; this is the discriminative claim.	No direct lipid prediction; NLRP3 mechanism is primarily immune. Lipoprotein subfraction differences between groups attributable to malonyl-CoA/CPT-1, not to NLRP3 [51].	ALDOB K108bhb-mediated mTOR inhibition demonstrated in cancer cell lines [58]; specific lipoprotein subfraction prediction in human dietary contexts is untested.	27-OHC predicted lower in committed KD via reduced hepatic cholesterol availability; co-directional lipid improvement is a speculative downstream inference. No human dietary oxysterol-lipoprotein data in IBD. Exploratory.	Bayesian SEM + LOO-CV; exploratory for H4
HMCRI (BA-MCY / immunomodulatory secondary BA sum)	Predicted lower in committed vs. intermediate groups; predicted higher in IBD/PSC regardless of dietary pattern (Coriobacteriaceae depletion as driver). BA-MCY FXR antagonist biology established [21]; HMCRI as a named	No prediction; BHB-direct NLRP3 mechanism does not engage BA-MCY counter-regulation.	No prediction; Kbhb-mTOR mechanism does not engage BA-MCY counter-regulation.	No prediction; oxysterol-LXR mechanism does not engage BA-MCY counter-regulation.	Bayesian SEM + LOO-CV; reference ranges established Stage 1

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Outcome Domain	H1: Bile Acid Signaling	H2: BHB-Direct NLRP3 Inhibition	H3: Kbhb-mTOR / Epigenetic	H4: Oxysterol-LXR (Exploratory)	Decision Rule
	construct is manuscript-derived and requires Stage 1 empirical validation. Reference ranges do not yet exist.				

H1 is the primary hypothesis; citations establish the mechanistic rationale generating each prediction, not observed dietary-group findings. H2 and H4 generate predictions directly testable in Stage 1. H3 is not directly testable cross-sectionally; BHB concentration provides an indirect proxy only, with formal mechanistic testing planned for Stage 3. H4 predictions are exploratory: supporting evidence derives from mouse models and neurological contexts; gut-specific demonstration has not been established. The hypotheses are not mutually exclusive; a finding consistent with H1 does not exclude contributions from H2, H3, or H4.

H1 enters Stage 1 with substantially stronger Bayesian prior weight for the specific Th17/Treg mechanism: four independent Nature-tier publications across two laboratories directly establish immunomodulatory bile acid species as Th17/Treg regulators in gut-relevant contexts [18,19,20,4]. H2, H3, and H4 have replicated mechanistic evidence in their respective contexts but have not been tested specifically for Th17/Treg balance. This asymmetry in prior support is distinct from the equivalence of dietary-group predictions, which are untested for all four hypotheses.

Discussion and Limitations

11. Discussion and Limitations

The Mediterranean dietary pole's co-directional lipid and immune improvements are supported by three IBD-specific RCTs and a prospective cohort of 271 newly diagnosed Crohn's disease patients [45–48]. For the ketogenic pole, favorable lipoprotein subfractions [86] and Foxp3⁺ Treg expansion [87] have each been documented under verified conditions but never simultaneously in IBD patients beyond the microbiome restructuring period; that simultaneous characterization is what this program proposes. No single pathway has been established as the upstream coordinator of these improvements. Bile acid signaling is the primary testable intermediate in Stage 1 because it is the only proposed mechanism measurable cross-sectionally that links dietary metabolic state to both output domains through a single analytical platform. Both committed patterns generate co-directional improvements through distinct mechanistic routes. Each produces coherent signaling across multiple interdependent receptor systems simultaneously; intermediate restriction does not. Bile acid signaling is the proposed coordinator of that coherence, not its proven cause.

The mechanistic core of this framework rests on a convergence of independently derived findings across laboratories and model systems. Three independent studies, published in Nature, showed that microbially generated bile acid species directly regulate the Th17/Treg balance through RORγt binding and mitochondrial ROS signaling [18,19,20], a finding subsequently extended to human gut bacteria, biosynthetic enzymes, and two independent IBD cohorts by Paik et al. [4]. The host counter-regulatory response through BA-MCY conjugate production [21] and the bile acid pool modification documented in the DIRECT-PLUS trial [44] further anchor the framework in human biology. New RCT-level evidence from Mediterranean diet–IBD trials (Seethaler 2025 [45], Haskey 2023 [46,47], Godny 2025 [48]) and ketogenic diet IBD preclinical data (Kong 2021 [53], Huang 2022 [52]) substantially strengthen the biological plausibility of the dietary intervention hypothesis in

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mucosal disease contexts. At the receptor pharmacology tier, the key constraints are built into the model rather than ignored: FXR's tissue-specific and post-translationally selective signaling [22,24], TGR5's opposing functional outputs across cell types [25,26,27], and S1PR2's context-dependent signaling (metabolic-protective under dietary conditions and pro-inflammatory in pathological injury contexts [28,29]) are all accommodated. Confidence in these mechanistic elements is substantially higher than confidence in the integrative dietary claim, which is why the latter is framed as a hypothesis generating a testable experimental program.

The hypothesis is most directly falsified if committed dietary groups do not differ in species-level immunomodulatory bile acid concentrations — specifically 3-oxoLCA, isoalloLCA, isoLCA, and isoDCA, despite differing in lipid and immune outcomes; such a finding would indicate that bile acid signaling is non-operative as the primary proposed intermediate, and BHB-direct NLRP3 inhibition [51] and Kbhb-mediated mTOR signaling [58] would become the primary candidates for Stage 3 prospective testing. The Stage 3 design is planned to distinguish these from bile acid-mediated effects.

Two sets of findings that appear contradictory warrant explicit interpretation. First, the regulatory withdrawal of obeticholic acid following REGENERATE Phase III [39] does not contradict dietary multi-receptor modulation; OCA achieved statistically significant fibrosis improvement but was withdrawn on risk-benefit grounds: atherogenic dyslipidemia, pruritus burden, and uncertain long-term outcomes. This is consistent with the interpretation that pharmacological activation of a single receptor at sustained supraphysiological concentrations produces a different benefit-risk profile than the coordinated physiological shifts generated by dietary commitment across multiple receptor systems simultaneously. Second, reports of transient CRP elevation during ketogenic dietary interventions [73] and increased small intestinal permeability in unverified ketosis conditions [17] are consistent with Prediction 4 rather than contradictory to the framework; both are predicted consequences of the microbiome transition period described in Section 5.2.

The gap this experimental program addresses is not simply the absence of BHB verification in prior studies, though that failure is pervasive. It is the absence of simultaneous multi-domain characterization: no study has yet measured verified metabolic state, the bile acid and SCFA metabolome, and Th17/Treg balance in the same subjects over a duration sufficient to capture committed-configuration steady-state biology. Each domain has been measured in isolation; the co-variation across all three has not. That co-variation is the specific test the framework requires, because the hypothesis is not that any one domain improves under committed dietary patterns but that the improvements are coupled and share a mechanistic basis. Correcting this three-failure design gap is the primary methodological contribution of the Stage 1 proposal.

The intermediate zone hypothesis, that the absence of verified ketosis in a nominally low-carbohydrate approach produces outcomes inferior to either committed pattern, carries no direct empirical support and is the most speculative element of the framework. Its importance lies in what has not been studied: the majority of studies enrolling self-described ketogenic diet participants have not verified sustained ketosis [13], and no study has characterized this population with respect to bile acid profiles, lipoprotein subfractions, Th17/Treg balance, or fecal calprotectin or endoscopic outcomes in IBD patients; these are the measurements most directly relevant to IBD disease activity.

Several limitations constrain interpretation. The majority of mechanistic evidence for bile acid immune regulation derives from mouse models; 6-hydroxylated bile acids (including muricholic acid species) constitute about half of the murine bile acid pool but are absent in humans, substantially altering the bile acid signaling landscape relative to human intestinal physiology [74,103]. This limitation is particularly consequential for the ketogenic diet mechanistic claims in this paper: the Kong 2021 ILC3 reduction data, the Ang 2020 microbiome restructuring kinetics, and the Huang 2022 BHB colonic mucosal findings all derive from murine models in which the secondary bile acid pool composition differs substantially from that of humans with IBD. The mechanistic inferences drawn from these studies, particularly regarding the bile acid signaling consequences of microbiome restructuring under ketogenic conditions, require prospective human validation before they can be applied to IBD patient populations with confidence. Within-person bile acid concentrations vary

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substantially across a single day, with coefficients of variation of 91–190% reported in healthy individuals. Single fasting timepoint sampling in Stage 1 captures mean-level group differences rather than temporal signaling stability. Establishing stability requires serial sampling within individuals, which is deferred to Stage 3. Temporal BHB dynamics, specifically the duration and frequency of above-threshold BHB elevation within a given participant, cannot be captured by the IOI design; IOI measures intra-individual carbohydrate variance rather than continuous BHB monitoring. Characterizing these dynamics requires continuous BHB measurement and is a Stage 3 requirement. Peripheral blood Th17/Treg ratios may not accurately reflect mucosal immune status in active IBD, where Treg sequestration to the mucosa reduces peripheral counts. Stage 1 Th17/Treg findings should be interpreted as systemic immune correlates rather than direct measures of mucosal status; fecal calprotectin serves as the complementary mucosal marker in the IBD arm. The IBD arm enrolls patients on stable maintenance therapy including biologic agents that directly modulate the Th17 axis under measurement; a null Th17/Treg difference in the IBD arm is therefore not interpretable without stratum-specific sensitivity analysis by maintenance therapy class.

Although Crohn's disease and ulcerative colitis differ in their upstream immunological architecture: Crohn's disease carries a predominantly Th1 signature and UC a modified Th2 signature, but both conditions converge on Th17 expansion and Treg insufficiency as the proximal mediator of mucosal injury, and it is at this convergence point that bile acid signaling is proposed to operate. The four receptor systems described capture the best-characterized pathways but omit others including PXR, CAR, and CHRM2/3. The BHB-HDAC inhibition mechanism has failed direct replication [56] and has been reconsidered in light of lysine β-hydroxybutyrylation as a distinct BHB-specific epigenetic program [57,58]. Lipid claims for strict ketosis rest on limited datasets, specifically the Virta Health cohort with 53% attrition and the KETO trial [15], whose longitudinal follow-up data [16] were subsequently retracted by JACC Advances due to methodological concerns; the cross-sectional KETO finding of no coronary plaque difference in the LMHR phenotype remains unretracted. The ketogenic dietary configuration's IBD-specific evidence base is substantially weaker than the Mediterranean diet's [45–48,52–54]; claims regarding the ketogenic configuration in IBD should be weighted accordingly pending prospective IBD-specific data. Mediterranean diet benefits operate through multiple parallel channels (polyphenols, MUFAs, fiber, and bile acids), with the bile acid contribution not yet isolated quantitatively in humans. The liver–brain–gut neural arc has not been independently replicated outside the originating laboratory [70]. Bile acid–mediated tolerance may suppress anti-tumor immunity in oncological contexts [33], requiring caution beyond metabolic and autoimmune disease. The microbiome–bile acid relationship is bidirectional, with BA-MCY counter-regulation [21] adding complexity to any unidirectional dietary model, and inter-individual microbiome variation will increase noise in Stage 1 comparisons, necessitating adequate cohort sizing. The group definitions also depend on individual BHB verification, because a given carbohydrate gram count does not reliably predict ketosis across individuals. BHB verification confirms the whole-diet metabolic state rather than circulating ketones as an effector: exogenous free-acid BHB supplementation does not replicate the ketogenic diet's anti-inflammatory effects in experimental colitis [85], reinforcing that the anti-inflammatory mechanisms the framework proposes operate through diet-induced microbiome remodeling rather than circulating ketone concentration per se; bile acids were not measured in that study and the specific mechanistic basis for the diet-vs-supplement divergence remains uncharacterized. Mediterranean diet adherence in the proposed Stage 1 design and in the cited cohort studies relies on self-reported dietary recall and adherence scoring; unlike the ketogenic arm, which uses BHB verification as an objective metabolic criterion, the Mediterranean diet arm lacks an equivalent biomarker confirmation step, and misclassification in this arm cannot be ruled out. The HMCRI construct and its Stage 1 analytical objectives are elaborated in Section 9.

The author declares no conflicts of interest. The author has ulcerative colitis and type 1 diabetes, conditions directly relevant to the biological pathways discussed.

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Figure Legends

Figure 1. The coupled bile acid receptor network. Dietary pattern-responsive bile acid signals converge on four receptor systems (FXR, TGR5, S1PR2, and RORγt) and an intersecting oxysterol-LXR sterol-immune axis to coordinately regulate hepatic lipid metabolism, NLRP3 inflammasome assembly, and intestinal Th17/Treg balance across three anatomical compartments: liver (left), gut lumen with microbial bile acid biotransformation (center), and the colonic immune compartment (right). The hepatic-vagal-colonic arc (dashed arrow) represents the neural relay identified by Teratani et al. [70]; this finding has not been independently replicated outside the originating laboratory and is shown as a putative connection only.

Figure 2. Predicted bile acid signaling environments across three dietary configurations. Three-column comparative schematic contrasting the committed ketogenic configuration (verified blood BHB ≥0.5 mM), the intermediate zone (carbohydrate restriction without verified ketosis), and traditional Mediterranean diet across five dimensions: microbiome state, dominant bile acid species, predicted RORγt signal, predicted Th17/Treg balance, and lipid metabolic profile. Green shading indicates coherent tolerogenic signaling; amber indicates uncertain or transitional states; the intermediate zone column carries a footnote annotation confirming that no human study has yet characterized this group for bile acid profiles, Th17/Treg ratio, or fecal calprotectin.

Figure 3. A staged experimental program for testing bile acid-mediated simultaneous dietary effects. Vertical flow diagram with three major nodes and two decision branches. Stage 1: cross-sectional bile acid metabolomics across four cohorts including a quiescent IBD arm. Stage 2: retrospective mediation analysis in stored biobank samples. Stage 3: prospective three-arm RCT with serial sampling at weeks 0, 2, 4, 8, and 12. A negative finding at any decision node does not nullify the empirical dietary hypothesis; it identifies the non-operative mechanism and redirects experimental design accordingly.

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Ethics Statement: This study does not involve human participants, animal subjects, or clinical trials and therefore did not require ethical approval.

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