Hypothesis

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Bile Acid Signaling as a Mechanistic Link Between Committed Dietary Patterns and Mucosal Immune Tolerance: A Hypothesis with a Staged Experimental Program

Running title: Dietary bile acid signaling and mucosal immune tolerance

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Abstract

Patients with inflammatory bowel disease and the allied cholangiopathy primary sclerosing cholangitis share a characteristic microbiome-metabolite signature: depletion of the secondary bile acid–producing bacteria responsible for generating immunomodulatory trace species — 3-oxolithocholic acid (3-oxoLCA), isoallolithocholic acid (isoalloLCA), isolithocholic acid (isoLCA), and isodeoxycholic acid (isoDCA). These species regulate the intestinal Th17/Treg balance through distinct mechanisms: 3-oxoLCA and isoLCA suppress Th17 differentiation through direct RORγt binding, while isoalloLCA promotes Foxp3+ Treg differentiation through mitochondrial reactive oxygen species signaling and Foxp3 CNS3 enhancer activation. Whether this depletion is a cause, consequence, or amplifier of mucosal inflammation remains incompletely resolved, but the convergence of microbiome, metabolomic, and immune data across independent IBD and PSC cohorts argues for a mechanistically important role. This paper proposes that committed dietary patterns — sustained ketosis (verified β-hydroxybutyrate ≥0.5 mM) and traditional Mediterranean diet — generate stable microbiome configurations capable of producing coherent multi-receptor bile acid signaling environments that coordinately promote mucosal immune tolerance. Intermediate carbohydrate restriction, defined by the absence of verified ketosis in individuals nominally following a low-carbohydrate approach, is proposed to generate signaling incoherence that defaults toward immunostimulation. The mechanistic framework operates through four primary receptor systems: hepatic FXR governing bile acid synthesis and lipogenesis; TGR5 stimulating GLP-1 secretion and suppressing NLRP3 inflammasome assembly in macrophages; S1PR2 mediating hepatic lipid metabolism gene regulation under dietary conditions; and RORγt responding to microbially generated 3-oxoLCA and isoLCA to suppress Th17 differentiation and expand Foxp3+ Treg populations. An oxysterol-LXR axis, operating through cholesterol oxidation products that modulate both Th17 and RORγt+ Treg subsets in an isoform-specific manner, is identified as a parallel sterol-immune regulatory system that intersects with the bile acid framework and provides a bidirectional link between cholesterol metabolism and mucosal immune tone. These receptor systems and sterol-derived signalling networks do not operate in isolation but form a coupled regulatory architecture in which the microbiome, bile acid pool, oxysterol landscape, hepatic lipid metabolism, and mucosal immune effectors are mutually constraining. The coherence of this network, this paper proposes, is the mechanism by which dietary commitment generates co-directional metabolic and immune outcomes.A staged experimental program is proposed — beginning with cross-sectional bile acid metabolomics across three dietary cohorts including patients with quiescent IBD — to test whether dietary pattern–specific bile acid profiles mediate co-directional immune outcomes and whether the intermediate zone represents a qualitatively distinct signaling environment relevant to IBD disease course.

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 characterised 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 confirms Th17 pathway activation as a proximate driver of pathological inflammation, not merely an epiphenomenon [1–3]. The parallel failure of broader immune suppression strategies to induce durable remission in substantial patient fractions points toward the importance of restoring tolerance mechanisms rather than simply attenuating inflammatory effectors.

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 IBD cohorts, with levels inversely correlating with IL-17-related host gene expression [4].

Second, fecal microbiome analysis in primary sclerosing cholangitis — a cholestatic autoimmune cholangiopathy in which 60–80% of affected patients carry concurrent IBD, predominantly ulcerative colitis — consistently demonstrates depletion of Eubacterium spp. and Ruminococcus obeum, genera encoding enzymatic machinery for secondary bile acid transformation, independent of IBD status [5]; Connelly et al. (2024) additionally confirmed lower fecal deoxycholic acid correlating with Blautia and Lachnoclostridium in PSC patients [6]. Mousa et al. (2021), analysing 400 PSC patients vs. 302 controls at Mayo Clinic, found markedly elevated primary-to-secondary bile acid ratios confirming deficient conversion at population scale [7].

The mechanistic inference is direct: depletion of the bacteria that produce immunomodulatory secondary bile acids removes a constitutive tolerogenic signal from the colonic mucosal environment, shifting the Th17/Treg equilibrium toward inflammation. 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, verified sustained ketosis produces an internally coherent metabolic profile including decreased small LDL particle number, reduced large VLDL particles, and improved inflammatory markers at one year [10,11]. Third, strict ketogenic diet produces dramatic microbiome restructuring with near-complete Bifidobacterium depletion through substrate deprivation and BHB-mediated growth inhibition, with confirmed downstream intestinal Th17 cell reduction via fecal transplant [12]. 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 suggests not a set of parallel unconnected effects but a coupled regulatory network — one in which bile acid signalling, oxysterol biology, microbiome ecology, and mucosal immune tone are mutually constraining and collectively responsive to dietary pattern.

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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 generalised 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 [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. The interpretation of the broader low-carbohydrate literature requires methodological contextualisation. 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 timing issue is particularly important in the context of IBD-relevant claims. Ang et al. [12] established that microbiome restructuring is a gradual ecological process requiring weeks to stabilise. A recent report of increased small intestinal permeability and elevated serum LPS in obese subjects following eight weeks of very low calorie ketogenic diet — conducted without blood BHB verification — is consistent with the transition-state inflammatory signal this framework predicts prior to microbiome stabilisation, rather than with the post-stabilisation committed configuration the hypothesis describes [17]. Whether eight weeks falls within or beyond the microbiome restructuring window in the IBD colon, where baseline dysbiosis may alter the stabilisation trajectory, cannot be determined from currently available data. A stabilisation window of four to eight weeks is a mechanistically plausible inference from the Ang et al. microbiome data, but this timeframe requires prospective verification in IBD-specific populations. This observation is therefore neither confirmatory nor refutatory with respect to the framework's IBD-relevant predictions; it underscores the need for longitudinal studies with verified ketosis and sufficient follow-up.

The central question is whether a shared upstream mechanism — bile acid signaling — coordinates the co-directional metabolic and immune improvements under committed dietary patterns, and whether the failure of intermediate carbohydrate restriction to achieve either committed configuration produces a signaling environment with clinical consequences for mucosal immune tone. This paper proposes bile acid signaling as the leading candidate mechanism.

Hypothesis Development

2. Bile Acid Signaling as the Proposed Mechanistic Link

Bile acids, once understood as passive fat-absorption facilitators, are now recognised 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 epimerisation producing immunomodulatory species including 3-oxolithocholic acid, isoallolithocholic acid, and isodeoxycholic acid [18–20]. Germ-free animals lack secondary bile acids entirely, confirming the microbiome's obligate role. 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 governing 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.

Four primary receptor systems transduce bile acid signals into metabolic and immune outputs across tissues. (VDR also responds to secondary bile acids, notably lithocholic acid, with tolerogenic effects on dendritic cells, but its contribution is not mechanistically developed in the present framework.) (i) Hepatic FXR (NR1H4) governs bile acid synthesis (CYP7A1/CYP8B1 repression via SHP), lipogenesis (SREBP-1c suppression), and lipoprotein remodelling. 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 use preferentially selective post-translational modifications: SUMO2 modification at K277 redirects individual FXR molecules from RXRα-dependent metabolic gene transactivation to NF-κB transrepression, while both programs coexist at the cellular level [24]. (ii) 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), blocking inflammasome assembly [25]. TGR5 activation also reduces oxidised LDL uptake and macrophage lipid loading in atherosclerosis models [26], representing the clearest example of cell-autonomous coupling of metabolic and immune functions in this receptor class. A dual-function controversy exists, however: protein kinase D phosphorylates the same NLRP3 residue (human Ser295) at the Golgi under different signaling contexts [27], producing the opposite outcome from PKA — PKD phosphorylation is activating, releasing NLRP3 from mitochondria-associated membranes to permit complex formation, whereas PKA phosphorylation suppresses assembly.

(iii) S1PR2, a receptor for conjugated bile acids, mediates hepatic lipid metabolism and immune cell trafficking. Under normal dietary conditions, conjugated bile acids signal through S1PR2 on hepatocytes via sphingosine kinase 2 (SphK2), upregulating lipid metabolism genes including SREBP-1c, FAS, and LDLR through nuclear S1P-mediated inhibition of HDAC1/2 [28]. This confers a metabolic-protective role distinct from S1PR2's behaviour in pathological inflammatory contexts. In pathological liver injury contexts, S1PR2 signaling in infiltrating macrophages via SphK1 promotes NLRP3 inflammasome priming and pro-inflammatory cytokine expression [29]. These represent distinct pathways — different ligands, cell types, and kinases — and the injury-context findings do not directly extrapolate to dietary signaling physiology.

(iv) 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]. Separately, NR4A1 has been identified as a downstream effector of bile acid-mediated Treg modulation through a bacterial metabolite distinct from isoalloLCA, independently of FXR and VDR [30]. Isolithocholic acid (isoLCA) is a structurally related but functionally distinct stereoisomer that also inhibits Th17 differentiation via RORγt binding. Paik et al. (2022) identified the specific human gut bacteria and 3α/3β-HSDH enzyme pairs responsible for the two-step biosynthetic conversion of LCA into 3-oxoLCA and then isoLCA, confirmed 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 IBD cohorts, with levels inversely correlating with IL-17-related host gene expression [4]. This represents the most direct human translational evidence currently available for the bile acid–Th17 regulatory axis in IBD-relevant cohorts. 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, 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 [19]. However, isoDCA's effects on FXR are cell-type dependent: Dong et al. (PNAS 2024) demonstrated potent FXR agonism in an FXRE-luciferase reporter assay in HEK293 cells cotransfected with FXR/RXR (EC50 = 4.384 μM) that suppresses Wnt signaling and colorectal cancer cell growth [31], illustrating the network's inherent capacity for opposing outputs depending on co-regulator context. Akagbosu et al. (2022) identified a novel RORγt+ antigen-presenting cell population ('Thetis cells') that induces microbiota-specific peripheral Treg differentiation via MHCII-dependent antigen presentation coupled with ITGB8-mediated activation of latent TGFβ1 — both mechanisms required on the same cell [32].

A further constraint concerns bile acid–mediated immune tolerance 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) operates additionally through ER stress via ATF6, IRE1α, and PERK signalling, and that blocking BAAT enhanced anti-PD-1 immunotherapy [33]. Importantly, 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-tumour 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.

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

Primary sclerosing cholangitis 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 centres 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]. Connelly 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].

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 in PSC colon tissue that was distinct from IBD alone and associated with dysplasia risk [35]. These findings are mechanistically consistent with the depletion of 3-oxoLCA and isoLCA whose RORγt-binding properties would be expected to suppress precisely this Th17-biased differentiation program. The causal contribution of specific gut pathobionts to hepatic Th17 responses in PSC has been directly demonstrated: 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 is one this framework identifies as a high-priority measurement target in the Stage 1 experimental design described below.

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PSC also illustrates the cholesterol–bile acid–immune interface in a clinically acute form. Bile acid deficiency in PSC impairs the FXR-mediated feedback repression of CYP7A1, which may increase hepatic cholesterol flux through the bile acid synthesis pathway and is proposed to elevate cholesterol intermediates and oxysterol precursor availability, though direct measurement of oxysterol profiles in PSC has not been reported. Three 2025 randomised controlled trials of atorvastatin adjunctive therapy in active ulcerative colitis — AlRasheed et al., Alarfaj et al., and Khrieba et al. — each demonstrated significant reductions in disease activity index, inflammatory cytokines (IL-6, TNF-α), and fecal calprotectin compared with mesalamine alone [36–38]. Alarfaj et al. additionally documented significant reduction in serum sphingosine-1-phosphate (S1P), a ligand for S1PR2 implicated in both macrophage activation and the hepatic lipid metabolism pathway described earlier [37]. These findings suggest that cholesterol metabolism and bile acid signaling intersect within the mucosal inflammatory circuit at a clinically modifiable node, and are discussed further in the mechanistic framework below.

Mechanistic Framework

3. The OCA Dissociation and Bile Acid Sequestrant Evidence

The strongest evidence against straightforward bile acid–mediated coupling comes from clinical pharmacology. In the REGENERATE Phase III trial (n=931), obeticholic acid improved hepatic fibrosis and reduced lobular inflammation while simultaneously producing an atherogenic lipid shift [39]. The FDA issued a Complete Response Letter in June 2023. The EMA revoked Ocaliva's conditional marketing authorisation for primary biliary cholangitis in 2024 based on the failed COBALT confirmatory trial (HR 1.01, 95% CI 0.68–1.51) [40]. No next-generation FXR agonist has resolved the atherogenic dyslipidaemia as of early 2026; resmetirom (a THR-β agonist, not an FXR agonist) was approved for MASH in March 2024, effectively supplanting the FXR agonist class [41]. INT-767, the only dual FXR/TGR5 agonist reported to have entered human trials (Phase 1, initiated 2015), was discontinued without published results.

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, though absolute risk reduction was 1.6 percentage points, statistical significance relied on a one-tailed test, and all-cause mortality was not reduced [42]. This trial has been superseded by the statin evidence base and is cited here primarily for its mechanistic implication: the present framework suggests that sequestrants may modulate both cholesterol metabolism and immune signaling by altering the bile acid pool — a speculative but testable interpretation.

4. Two Committed Dietary Configurations and Their Bile Acid Environments

4.1. 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 — 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] — the first RCT to demonstrate that Mediterranean diet-induced bile acid compositional shifts mediate improvements in a gut-specific endpoint — distinct from DIRECT-PLUS's demonstration that baseline bile acid levels modify the magnitude of cardiometabolic response to Mediterranean diet. Haskey et al. (2023, IRJC trial, n=28 quiescent UC, randomised) 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]. Godny et al. (2025, prospective cohort, n=271 newly diagnosed CD patients) found MedDiet adherence inversely correlated with cholic acid, chenodeoxycholic acid, and pro-inflammatory kynurenines, while correlating positively with Faecalibacterium and SCFAs, and inversely with CDAI, fecal calprotectin, and CRP [48].

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 the best-supported mechanistic channel 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 neither depend on the bile acid hypothesis nor require it for clinical justification, but their co-occurrence with immune improvements is precisely the empirical observation this framework sets out to explain mechanistically.

4.2. Committed Ketogenic Configuration

Sustained carbohydrate restriction below 35 g/day depletes malonyl-CoA, disinhibits CPT-1, and establishes maximal hepatic β-oxidation with systemic BHB reaching 1–5 mM. The microbiome undergoes dramatic restructuring: Bifidobacterium is markedly depleted through substrate deprivation and direct BHB growth inhibition, reducing intestinal Th17 cells [12]. Mean laboratory BHB at one year in the Virta Health cohort was 0.30 mmol/L across the full cohort [75], consistent with a proportion of participants not achieving the framework's ≥0.5 mM committed-ketosis threshold — an important caveat for interpretation. Li et al. (2024) identified specific serum taurine-conjugated bile acid species (TDCA and TUDCA) elevated under ketogenic conditions in mice and validated in human cohorts (n=416 observational; n=25 interventional), attributing the shift to decreased BSH-coding Lactobacillus murinus reducing bile acid deconjugation [50]. TUDCA is a characterised TGR5 agonist and cytoprotective bile acid with demonstrated protective effects in ER stress contexts. BHB directly inhibits the NLRP3 inflammasome by preventing potassium efflux and ASC oligomerisation — a finding replicated across laboratories, independent of GPR109A, AMPK, and autophagy [51].

In the IBD-specific context, two complementary findings support the ketogenic configuration's plausibility. First, Huang et al. (2022) demonstrated that BHB levels are significantly reduced in colonic mucosa of both UC and CD patients and inversely correlate with disease activity, with rectal BHB enema ameliorating DSS colitis via STAT6-dependent M2 macrophage polarisation independent of gut microbiota [52]. Second, Kong et al. (2021) showed that ketogenic diet alleviates DSS colitis by reducing colonic RORγt+CD3− ILC3s and inflammatory cytokines (IL-17α, IL-18, IL-22) through microbiome-dependent mechanisms, confirmed by fecal microbiota transfer into germ-free mice [53]. The ILC3 reduction finding is particularly notable given ILC3s' role in sustaining colonic Th17 programs in IBD.

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]. These data are insufficient for clinical

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inference but provide signal warranting prospective investigation. Separately, 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 independent of any immunological claim. That these improvements occur alongside immune remodelling is the empirical co-occurrence motivating the shared-mechanism hypothesis; each dimension retains clinical significance whether or not they prove mechanistically linked.

On BHB epigenetics: the widely cited claim that BHB inhibits class I HDACs (Shimazu et al. 2013 [55]) was directly challenged by Chriett et al. (2019), who could not detect BHB HDAC inhibitory activity across multiple cell types [56]. Subsequent research has converged on lysine β-hydroxybutyrylation (Kbhb) as the predominant BHB-specific epigenetic modification. Xie et al. (2016) identified 44 histone Kbhb sites including H3K9bhb [57]. Qin et al. (2024) provided comprehensive multi-omic validation, demonstrating that ketogenic diet reshapes metabolism primarily through Kbhb — including ALDOB K108bhb inhibiting mTOR signaling — rather than classical HDAC inhibition [58]. This is not HDAC inhibition but a distinct, BHB-specific epigenetic program that butyrate cannot produce.

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

Given that committed dietary patterns each generate stable microbiome configurations and correspondingly stable bile acid signaling environments, what happens at intermediate carbohydrate restriction (50–150 g/day) or with vacillating compliance? The mechanistic logic suggests that periodic carbohydrate intake would generate rapid malonyl-CoA elevation (documented at approximately 2.7-fold within hours of hyperglycaemia with hyperinsulinaemia [59]) that intermittently inhibit CPT-1, preventing the establishment of sustained β-oxidation. The microbiome would occupy a transitional community state — neither the Bifidobacterium-depleted KD configuration nor the diverse saccharolytic MedDiet configuration.

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. 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. 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 fibre, 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 in this paper (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 characterised for Th17/Treg ratio.

The Seidelmann et al. (2018) cohort study found a U-shaped mortality curve across carbohydrate intake (n=432,179), with the nadir at 50–55% carbohydrate energy — approximately 250–275 g/day at a 2,000 kcal intake — and monotonically increasing mortality risk below 40% of energy [60]. The curve does not show a non-monotonic intermediate peak consistent with a discrete intermediate signaling zone; it is better interpreted as reflecting diet quality confounding, as Angelotti et al. (2024) found in NHANES data (1999–2018) that restricted carbohydrate diets below 45% energy were not associated with increased all-cause mortality risk (HR 0.98, 95% CI 0.87–1.11), while cardiovascular mortality trended higher (HR 1.20, 95% CI 0.96–1.49) [61]. Neither dataset characterises a mechanistically distinct intermediate zone; whether one exists remains an empirical question the Stage 1 design addresses directly.

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

Bile acids are sterol-derived signaling molecules, and their biosynthesis from hepatic cholesterol via CYP7A1 and CYP8B1 is regulated by the same nuclear receptor systems — particularly liver X receptor (LXR) — that govern cholesterol oxidation product (oxysterol) signaling. This biochemical connectivity creates a bidirectional regulatory circuit: dietary patterns that alter bile acid pool composition also alter the hepatic cholesterol intermediate landscape, and the oxysterols generated from those intermediates independently modulate mucosal immune balance through mechanisms that parallel and intersect with the bile acid framework.

Parigi et al. (2021, Mucosal Immunology) provided the most mechanistically relevant demonstration in gut-specific context, showing that LXR deficiency increases both mesenteric lymph node Th17 cells and RORγt+ Tregs through isoform-specific mechanisms: LXRα modulated only Th17 cells, while LXRβ regulated both Th17 and RORγt+ Tregs through signaling in CD11c+ myeloid cells [62]. Jacobse et al. (2023) extended this by demonstrating that IL-23R signaling downregulates LXR target genes in colonic Tregs, impairing their stability and function — with an LXR inverse agonist decreasing colonic Treg frequency, and human scRNA-seq of Crohn's ileal lamina propria confirming IL-23R expression on Tregs [63]. These findings place LXR at the intersection of 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, with the RORγt inhibitor SR1001 reversing this effect [64,65]. 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 — though whether they compete for the same binding site or act through distinct conformational mechanisms has not been established. 25-hydroxycholesterol (25-HC) acts through GPR155 on macrophage lysosomes, activating AMPK and driving immunosuppressive metabolic reprogramming [66]. 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 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 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.

The statin evidence in IBD provides indirect clinical support for this cholesterol–immune node. The Alarfaj et al. 2025 atorvastatin RCT found significant reductions in serum S1P alongside reduced

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IL-6 and TNF-α in UC patients — directly linking HMG-CoA reductase inhibition to the S1PR2/SphK1 pro-inflammatory pathway in the colonic macrophage context [37]. Mendelian randomisation analysis found that HMGCR-mediated LDL-C lowering (the statin mechanism) did not increase IBD risk, while PCSK9-mediated LDL-C lowering paradoxically did — suggesting that statins' IBD benefit involves pleiotropic anti-inflammatory mechanisms, potentially including oxysterol and bile acid precursor modulation, beyond simple cholesterol reduction [69]. These data do not establish dietary cholesterol modulation as the operative mechanism, but they confirm that interventions affecting the cholesterol synthesis pathway alter IBD-relevant inflammatory signals at clinically meaningful magnitudes.

6. Systems-Level Coordination: the Hepatic-Vagal-Colonic Arc

Distributed coordination between metabolism and immunity is anatomically grounded. Teratani et al. discovered a liver–brain–gut neural arc in which hepatic vagal sensory afferents detect portal blood metabolites — the specific chemical signal was not identified in the original study, though bile acids are plausible candidates given their portal concentration dynamics — and relay through the nucleus tractus solitarius to generate efferent vagal output maintaining the colonic Treg cell niche [70]. This finding has not yet been independently replicated outside the originating Kanai laboratory at Keio University; the technically demanding lateralised (left vagal) microsurgical procedures may explain the absence of independent replication attempts, which is distinct from failed replication. Zhu et al. (2024) identified an opposing neural circuit: TRPV1+ dorsal root ganglia nociceptor neurons suppress RORγt+ Tregs via CGRP-RAMP1 signaling [71], demonstrating bidirectional neural regulation of gut Tregs.

GLP-1, secreted by L-cells in response to bile acids (TGR5) and SCFAs (GPR43), serves as a complementary humoral coordination molecule, suppressing hepatic lipogenesis and modulating innate immune tone. GLP-1 analogue therapy reduces inflammatory cytokine production in immune cells, providing additional evidence for a link between this bile acid–driven secretory axis and immune regulation [72]. Dietary patterns that shift the bile acid pool therefore engage three distinct coordination layers simultaneously: receptor-mediated signalling through FXR, TGR5, S1PR2, and RORγt; neural relay through the hepatic vagal arc; and humoral modulation through GLP-1 and the enterohepatic circulation. That each layer is responsive to bile acid pool composition is the mechanistic basis for the integrative argument developed in the Discussion.

Experimental Program

7. A Staged, Affordable Experimental Program

Rather than proposing a single definitive trial, this paper outlines a staged program designed to be practical, affordable, and sequentially informative. 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.

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); persons on documented Mediterranean diet (≥12 weeks, dietary recall–confirmed); persons consuming intermediate carbohydrate restriction (50–150 g/day) without verified ketosis; and patients with quiescent IBD (Mayo score ≤1 for UC, Harvey-Bradshaw ≤4 for CD, on stable maintenance therapy) consuming either committed or intermediate dietary patterns. Perform targeted bile acid metabolomics (including 3-oxoLCA, isoalloLCA, isoLCA, isoDCA, BA-

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MCY conjugates, conjugated and unconjugated primary and secondary species), oxysterol profiling (27-OHC, 25-HC, 7α,25-diHC), NMR lipoprotein subfraction analysis, and basic immune panel (Th17/Treg ratio by flow cytometry, hsCRP, IL-6, fecal calprotectin for IBD arm). Test whether bile acid profiles differ between 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 trace bile acid species relative to those on intermediate patterns. Estimated cost: $200,000–$400,000 at academic core facility rates.

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 modelling. 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 pre-specified 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.

8. Testable Predictions

The framework generates specific predictions that distinguish it from the null hypothesis of independent metabolic and immune effects. Prediction 1: co-directional changes in lipoprotein subfractions and Th17/Treg ratio will differ systematically across the three dietary groups, with committed patterns outperforming intermediate restriction on both measures. If confirmed, this correlation will be substantially attenuated when bile acid profile is included as a mediating variable in structural equation modelling. 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 FXR signaling balance. Prediction 4: during dietary transition (weeks 1–4), a transient inflammatory elevation should coincide with microbiome restructuring and should be most pronounced in the intermediate arm, where restructuring is predicted to be incomplete. 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.

Discussion and Limitations

9. Discussion and Limitations

The framework presented here is deliberately integrative because the underlying biology operates as a network of coupled feedback loops rather than isolated linear pathways. Bile acid synthesis begins with cholesterol — the same sterol pool that generates immunomodulatory oxysterols acting through LXR on Th17 and RORγt$^+$ Treg subsets — and the microbiome both sculpts and is reciprocally shaped by the bile acid and oxysterol pools it helps produce. Immune activation modulates hepatic CYP7A1 expression and cholesterol efflux; S1PR2 and FXR signalling couple hepatic lipid metabolism to mucosal immune tone; and host counter-regulatory BA-MCY conjugates [21] close additional loops that constrain the system's response to dietary perturbation. The clinical implication is that interventions targeting any single receptor or metabolite in isolation are likely to produce incomplete or transient effects — a prediction consistent with the failure of pharmacological FXR agonism to replicate the co-directional metabolic and immune shifts generated by dietary commitment across multiple receptor systems simultaneously [39]. It remains speculative whether the intermediate dietary zone constitutes a distinct and reproducible signalling configuration or simply reflects the expected variance of an incompletely committed system; the Stage 1 cross-sectional design is structured precisely to test that distinction. What the network architecture does suggest, with greater confidence, is that durable restoration of mucosal immune tolerance through dietary means will require stable commitment to a pattern that generates coherent input across the receptor systems described — not partial modulation of any one node.

The mechanistic core of this framework rests on a convergence of independently derived findings across laboratories and model systems. Three independent Nature papers established that microbially generated bile acid species directly regulate the Th17/Treg balance through RORγt binding and mitochondrial ROS signaling [18–20], 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 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 [25–27], 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 would be most directly falsified if the proposed cross-sectional study revealed that 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 establish that bile acids are not the coordinating mechanism, even if the co-directional empirical observation retained independent scientific value.

Two sets of findings that appear contradictory warrant explicit interpretation. First, the failure of obeticholic acid in REGENERATE Phase III [39] does not contradict dietary multi-receptor modulation; it demonstrates that pharmacological activation of a single receptor at supraphysiological concentrations produces different outcomes than the coordinated physiological shifts generated by dietary commitment across multiple receptor systems simultaneously. Second, reports of transient CRP elevation during ketogenic dietary interventions, such as that observed by Hengist et al. [73], are consistent with Prediction 4 rather than contradictory to it. A recent report of increased small intestinal permeability and elevated serum LPS in obese subjects following eight

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weeks of very low calorie ketogenic diet conducted without blood BHB verification [17] is consistent with the same transition-state dynamics: the Ang et al. data [12] establish that Bifidobacterium depletion and microbiome remodelling take weeks to stabilise, and the framework predicts precisely this pattern of transient barrier disruption during the period of ecological restructuring. A stabilisation window of four to eight weeks is a mechanistically plausible inference from the microbiome kinetics data, but the specific timeline — particularly in the IBD colon where baseline dysbiosis may alter stabilisation dynamics — requires prospective characterisation.

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 characterised this population with respect to bile acid profiles, lipoprotein subfractions, Th17/Treg balance, or, crucially, fecal calprotectin or endoscopic outcomes in IBD patients.

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 approximately half or more of the murine bile acid pool but are absent in humans, limiting direct extrapolation [74]. Although Crohn's disease and ulcerative colitis differ in their upstream immunological architecture — Crohn's disease carrying a predominantly Th1 signature and UC a modified Th2 signature — 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-characterised 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, notably the Virta Health cohort with 53% attrition and the KETO trial with retracted longitudinal data. Mediterranean diet benefits operate through multiple parallel channels — polyphenols, MUFAs, fibre, 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 immune tolerance may suppress beneficial anti-tumour immunity [33], with UDCA protective and LCA/DCA immunosuppressive in oncological contexts, requiring caution in extending the framework beyond metabolic and autoimmune disease. The microbiome–bile acid relationship is bidirectional, and host BA-MCY counter-regulation [21] adds complexity to any unidirectional dietary model. Inter-individual microbiome variation substantially exceeds dietary effects in controlled feeding studies, and the qualitative group definitions proposed here depend on individual BHB verification because a given carbohydrate gram count does not reliably predict ketosis across individuals. The oxysterol-LXR-Treg circuit described in Section 5a is supported by mechanistic primary data in gut-relevant contexts, but its dietary modulation under committed KD or Mediterranean diet conditions has not been measured in humans; oxysterol profiling should be incorporated into Stage 1 as a priority.

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