Hypothesis

Bile Acid Signaling as a Mechanistic Link Between Committed Dietary Patterns, Lipid Metabolism, and Immune Tolerance: An Integrative Hypothesis with a Staged Experimental Program

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Bile Acid Signaling as a Mechanistic Link Between Committed Dietary Patterns, Lipid Metabolism, and Immune Tolerance: An Integrative Hypothesis with a Staged Experimental Program

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Abstract

An empirical pattern recurs across the dietary intervention literature: committed dietary patterns—sustained ketosis (<35 g carbohydrate/day with verified β-hydroxybutyrate ≥0.5 mM) and Mediterranean diet—each improve inflammatory markers and, under verified conditions, produce favorable or non-atherogenic lipid profiles. Intermediate carbohydrate restriction (50–150 g/day, or vacillating compliance without sustained ketosis) may not achieve either. Simultaneously, strict ketogenic diets produce dramatic gut microbiome restructuring, including near-elimination of *Bifidobacterium adolescentis* and expansion of *Akkermansia muciniphila*. This paper proposes that microbiome-mediated bile acid signaling is the mechanistic link connecting these observations. The microbiome generates the majority of bile acid chemical diversity through deconjugation, dehydroxylation, and epimerization of host-synthesized primary species, while the host simultaneously produces counter-regulatory bile acid conjugates. Dietary patterns that produce stable microbiome configurations therefore also produce stable bile acid signaling environments that coordinate, through multiple receptors including FXR, TGR5, S1PR2, VDR, and RORγt, both lipid metabolic and immune outcomes across organ compartments. This coordination is distributed across tissues and receptors with sometimes opposing outputs, not tightly coupled through a single molecular effector. The hypothesis must account for established findings that constrain it: FXR's metabolic and anti-inflammatory programs use mutually exclusive post-translational modifications within single cells; the FXR agonist obeticholic acid improved hepatic inflammation while worsening atherogenic lipid profiles in Phase III trials; individual bile acid species exert cell-type-dependent effects on the same receptor; and the most potent bile acid immune tolerance pathways bypass FXR and TGR5 entirely. Moreover, bile acid–mediated immune tolerance may simultaneously suppress beneficial anti-tumor immunity in certain tissue contexts. Despite these constraints, the framework generates testable predictions and a staged, affordable experimental program is proposed. **Take-home message:** Bile acids are not passive fat-absorption facilitators but a multi-receptor signaling network through which committed dietary patterns may simultaneously coordinate lipid metabolism and immune tolerance—explaining why these outcomes co-vary under dietary intervention and why intermediate restriction fails at both.

Keywords: bile acids; FXR; TGR5; S1PR2; VDR; RORγt; gut microbiome; ketogenic diet; Mediterranean diet; immune tolerance; Th17; Treg; NLRP3; lipid metabolism; enterohepatic circulation; β-hydroxybutyrylation

1. The Empirical Observation

Three empirical patterns motivate this hypothesis. First, committed Mediterranean diet reduces cardiovascular events by approximately 30% (PREDIMED, n=7,447 [1]), a finding independently confirmed in secondary prevention by CORDIOPREV (n=1,002; HR 0.719, 95% CI 0.541–0.957, fully

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adjusted model [2]), with simultaneous improvements across lipid, inflammatory, and glycemic domains. Second, verified sustained ketosis produces a distinct but also internally coherent metabolic profile. Virta Health's continuously monitored cohort (BHB ≥0.5 mM) showed decreased small LDL-P, increased LDL particle size, decreased large VLDL-P, and improved hsCRP at 1 year [3], with 5-year follow-up confirming sustained triglyceride and HDL improvements among completers [4]—though survivorship bias is significant (262→122 completers, 53% attrition), the study is non-randomized, and no independent group has replicated the lipid subfraction findings. Third, Ang et al. demonstrated that strict ketogenic diet produces near-complete microbiome restructuring, depleting *Bifidobacterium adolescentis* through substrate deprivation and direct BHB-mediated growth inhibition, with confirmed downstream Th17 cell reduction via fecal transplant [5].

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 [6]. BHB testing compliance declined to 63% even in well-designed trials over 10 weeks [7]. 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 [8]. However, longitudinal follow-up of 100 LMHR individuals (Soto-Mota et al., 2025) reported a median non-calcified plaque volume increase of 18.9 mm^3/year (mean 31.5 mm^3/year, reflecting high individual variability)—substantially higher than rates observed in general population cohorts. Significant caveats apply: no control arm was included in the longitudinal analysis, the pre-registered primary outcome was acknowledged by the authors as inadequately reported, and JACC Advances has issued an Expression of Concern regarding this publication [9]. The lipid and atherosclerosis claims in this paper are therefore stated with appropriate uncertainty: whether strict ketosis (<35 g/day, sustained BHB ≥0.5 mM) consistently produces favorable cardiovascular outcomes across populations remains an open and actively contested question.

The observation that demands explanation is not any single finding but the co-occurrence: committed dietary patterns appear to improve both lipid profiles and inflammatory markers simultaneously, while intermediate restriction may fail at both. If these were independent biological systems, co-directional changes under dietary intervention would be coincidental. The question is whether a shared upstream mechanism coordinates them.

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 both lipid metabolism and immune regulation. 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 [10–12]. 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 [13]. The bile acid signaling landscape is therefore shaped by both microbial transformation (generating FXR agonists) and host counter-regulation (generating FXR antagonists), with diet influencing the balance.

Five receptor systems transduce bile acid signals into metabolic and immune outputs across tissues. (i) 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 [14]. FXR's metabolic and anti-inflammatory programs use mutually exclusive post-translational modifications: SUMO2

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modification at K277 enables NF-κB transrepression but blocks RXRα heterodimerization for metabolic gene transactivation [15]. (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 and reducing oxidized LDL uptake [16,17]. TGR5 in macrophages represents the clearest example of cell-autonomous coupling of metabolic and immune functions, though a dual-function controversy exists: protein kinase D phosphorylation at the same NLRP3 site may activate rather than inhibit the inflammasome under different signaling contexts [18]. (iii) S1PR2, the primary receptor for conjugated bile acids, mediates hepatic inflammatory signaling and immune cell trafficking; S1PR2 signaling is predominantly pro-inflammatory in hepatic and intestinal contexts (promoting M1 macrophage polarization), though tissue-dependent exceptions exist—isoLCA acts anti-inflammatory through S1PR2 in airway macrophages [42]—contrasting TGR5's anti-inflammatory effects and illustrating the network's inherent capacity for opposing outputs [19]. (iv) Microbially generated 3-oxolithocholic acid directly binds RORγt to suppress Th17 differentiation [10]. (v) Isoallolithocholic acid promotes Foxp3+ Treg differentiation through mitochondrial reactive oxygen species signaling and the Foxp3 CNS3 enhancer, with NR4A1 identified as the downstream effector, independently of FXR and VDR [10,20].

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 [11]. However, isoDCA's effects on FXR are cell-type dependent: Dong et al. (PNAS 2024) demonstrated potent FXR agonism in intestinal epithelial cells (EC_{50} = 4.384 μM) that suppresses Wnt signaling and colorectal cancer cell growth [43], illustrating the network's inherent capacity for opposing outputs depending on co-regulator context. This duality exemplifies why single-receptor pharmacology (Section 3) fails where multi-receptor dietary modulation may succeed. Akagbosu et al. (2022) identified a novel RORγt+ antigen-presenting cell population ("Thetis cells") that induces microbiota-specific peripheral Treg differentiation via MHCII—rather than conventional DCs—as the key tolerogenic mechanism in the gut [21].

An important constraint: bile acid immune tolerance has a dark side. Varanasi et al. (2025) demonstrated that conjugated and secondary bile acids (LCA, DCA) impair CD8+ T cell function in hepatocellular carcinoma through oxidative and ER stress, and that blocking BAAT (bile acid-CoA:amino acid N-acyltransferase) enhanced anti-PD-1 immunotherapy [22]. Importantly, these effects are species-specific: 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—and different dietary patterns would shift the pool toward different species with divergent immunological consequences. The framework proposed here applies to metabolic and autoimmune contexts; its extension to oncology requires additional consideration of this trade-off.

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 [23]. The FDA issued a Complete Response Letter in June 2023. The EMA revoked Ocaliva's conditional marketing authorization for primary biliary cholangitis in 2024 based on the failed COBALT confirmatory trial (HR 1.01, p=0.954); Intercept had separately withdrawn its EMA NASH marketing authorization application in December 2021 [24]. No next-generation FXR agonist has resolved the lipid dyslipidemia problem 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 [25]. No dual FXR/TGR5 agonist has yet reached human clinical trials, and the LDL-raising class effect of FXR agonism remains a translational barrier that dietary multi-receptor activation may circumvent.

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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 the absolute risk reduction was 1.6 percentage points (7.0% vs. 8.6%), statistical significance relied on a one-tailed test, and all-cause mortality was not reduced [26]. This trial is now primarily of historical significance, superseded by the statin evidence base, but 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 GPR43/GPR109A on colonic Tregs driving Foxp3+ expansion [27]. The diverse microbiome generates secondary bile acids including isoDCA that promotes Treg differentiation via FXR antagonism on dendritic cells [11]. The DIRECT-PLUS trial (Gao et al., n=284) demonstrated that baseline fecal bile acid levels significantly modified the beneficial cardiometabolic effects of Mediterranean diet intervention [28]—the first RCT evidence that baseline bile acid profiles significantly modify the cardiometabolic response to dietary intervention. However, 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.

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 adolescentis* is depleted through substrate deprivation and direct BHB growth inhibition, reducing intestinal Th17 cells [5]. Li et al. (2024) demonstrated in combined observational (n=416) and interventional (n=25) human studies that ketogenic diets increase serum TDCA and TUDCA by depleting BSH-coding *Lactobacillus murinus* [29]—the first direct evidence linking ketogenic diets to specific bile acid profile changes in humans. BHB directly inhibits the NLRP3 inflammasome by preventing potassium efflux and ASC oligomerization—a robust finding replicated across laboratories, independent of GPR109A, AMPK, and autophagy [30].

On BHB epigenetics: from HDAC inhibition to β-hydroxybutyrylation. The widely cited claim that BHB inhibits class I HDACs (Shimazu et al. 2013 [31]) was directly challenged by Chriett et al. (2019), who could not detect BHB HDAC inhibitory activity across multiple cell types [32]. The field has since pivoted decisively to lysine β-hydroxybutyrylation (Kbhb) as the dominant BHB epigenetic mechanism. Xie et al. (2016) identified 44 histone Kbhb sites including H3K9bhb [33]. 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 [34]. Over 5,000 Kbhb modification sites on non-histone proteins have now been catalogued across multiple proteomics studies, with p300 identified as the primary writer and HDAC1–3 and SIRT1–3 as erasers. This is not HDAC inhibition but a distinct, BHB-specific epigenetic program that butyrate cannot produce. The present framework relies on Kbhb rather than contested HDAC inhibition as the BHB epigenetic mechanism.

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

If 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

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periodic carbohydrate intake would generate malonyl-CoA oscillations (documented at 2.7-fold increases within hours of hyperglycemia [35]) that intermittently inhibit CPT-1, preventing the establishment of sustained β-oxidation. The microbiome would occupy a transitional community.

This paper explicitly distinguishes two levels of claim. **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. This is the most speculative element of the present framework: no human study has yet performed bile acid metabolomics across a carbohydrate restriction gradient (strict ketosis <35 g/day vs. moderate 50–150 g/day vs. standard diet >200 g/day). Existing two-group comparisons (Li et al. 2024 [29]) and mouse data hint at possible non-linear bile acid responses to carbohydrate restriction, making the committed-vs-intermediate distinction an important empirical question that Stage 1 of the proposed program will directly test. The Seidelmann et al. mortality data (n=432,179) showing a U-shaped curve with minimum at 50–55% carbohydrate energy [36] is more consistent with continuous dose-response than with discrete pathological states, though 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), though cardiovascular mortality trended higher (HR 1.20, 95% CI 0.96–1.49)—suggesting that the Seidelmann U-curve's low-carbohydrate arm may partly reflect confounding by diet quality [37]. Whether the intermediate zone is merely suboptimal or constitutes a qualitatively different signaling environment is an empirical question this paper cannot answer but can motivate.

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 (including bile acids), relay through the nucleus tractus solitarius, and generate efferent vagal output maintaining the colonic Treg cell niche [38]. This finding has not yet been independently replicated outside the originating Kanai laboratory at Keio University; the technically demanding lateralized (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 (DRG) nociceptor neurons—distinct from the vagal afferents in Teratani's circuit—suppress RORγt+ Tregs via CGRP-RAMP1 signaling [39], 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, simultaneously suppressing hepatic lipogenesis and promoting Treg differentiation [40].

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.

Stage 1: Cross-sectional bile acid profiling across dietary patterns. Recruit three 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), and persons consuming a standard Western diet with intermediate carbohydrate intake. Perform targeted bile acid metabolomics (including 3-oxoLCA, isoalloLCA, isoDCA, BA-MCY conjugates, conjugated/unconjugated primary and secondary species), NMR lipoprotein subfraction analysis, and basic immune panel (Th17/Treg ratio by flow cytometry, hsCRP, IL-6). Test whether bile acid profiles differ between groups and correlate with simultaneous lipid and immune outcomes. Estimated cost: $200,000–$400,000.

Stage 2: Bile acid mediation analysis in existing trial biobanks. The PREDIMED, DIRECT-PLUS, and Virta Health cohorts have stored biological samples. Retrospective bile acid metabolomics

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on stored samples, combined with existing lipid and inflammatory marker data, could test whether bile acid profile changes mediate the 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 keto 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. Given documented inter-individual microbiome variation (typically explaining 50–85% of compositional variance versus 1.5–10% for diet in controlled feeding studies), a crossover design with ≥100 participants or parallel-arm design with 200–500 participants would likely be required for 80% power to detect medium-sized mediation effects in structural equation modeling. 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:** changes in lipoprotein subfractions and Th17/Treg ratio will be positively correlated within individuals across dietary arms, and this correlation will be substantially attenuated when bile acid profile is included as a mediating variable. **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) will differ between dietary groups and contribute to the FXR signaling balance. **Prediction 4:** during dietary transition (weeks 1–4), a transient inflammatory spike should coincide with microbiome restructuring, most pronounced in the intermediate arm.

9. Limitations

This hypothesis carries several limitations. The majority of mechanistic evidence for bile acid immune regulation comes from mouse models, and murine bile acid composition differs fundamentally from human: muricholic acids constitute 35–50% of the mouse bile acid pool but are absent in humans [41]. The five-receptor model captures the best-characterized pathways but omits others (PXR, CAR, CHRM2/3). The BHB-HDAC inhibition mechanism has failed direct replication [32] and has been superseded by Kbhb as the dominant BHB epigenetic mechanism [33,34]. The lipid claims for strict ketosis rely on limited datasets (Virta Health with 53% attrition, KETO trial with contested longitudinal data) because the broader "low-carb" literature does not adequately define or verify the dietary intervention. The "signaling incoherence" concept for intermediate restriction has zero direct empirical support and represents the most speculative element of this framework. Mediterranean diet benefits operate through multiple parallel channels (polyphenols, MUFAs, fiber, bile acids) with the bile acid contribution not yet isolated quantitatively in humans. The liver–brain–gut neural arc has not been independently replicated. And bile acid–mediated immune tolerance may suppress beneficial anti-tumor immunity [22], with species-specific effects (UDCA protective, LCA/DCA immunosuppressive) requiring caution in extending this framework to oncology contexts.

The microbiome–bile acid relationship is bidirectional, and the discovery of host BA-MCY counter-regulation [13] adds a further layer of complexity. Inter-individual microbiome variation substantially exceeds dietary effects in controlled feeding studies, yet functional convergence in bile acid metabolism may preserve signaling coherence despite taxonomic differences—a possibility that requires direct testing. The enterohepatic circulation continuously recycles modified bile acids through the hepatobiliary system. Disentangling cause from effect in these feedback loops requires the staged experimental program described above.

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10. Version History

Version 1 (retracted) proposed tight molecular coupling through FXR/SHP. Versions 2–4 incorporated successive adversarial corrections: receptor assignments, lipid claim hedging, 2025–2026 temporal validation. Versions 5–6 corrected specific citation errors identified in claim-by-claim audits. Version 7 was the editorially polished submission version. Version 8 (current) incorporates targeted revisions from a second adversarial review cross-checked by an independent AI system: isoDCA cell-type-dependent FXR pharmacology (Dong et al. PNAS 2024 [43]), explicit acknowledgment of the absence of human bile acid gradient studies across carbohydrate restriction levels, statistical power requirements for the proposed Stage 3 trial, the FXR agonist translational barrier, UDCA's species-specific protective effects in anti-tumor immunity, and microbiome inter-individual variance.

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Use of Artificial Intelligence: The author used Claude (Anthropic, 2026) as the primary AI assistant for systematic literature search, iterative adversarial analysis of the hypothesis (which identified receptor assignment errors, the BHB-HDAC replication failure, isoDCA cell-type duality, and other weaknesses corrected across successive versions), temporal validation of claims against 2025–2026 literature, and manuscript preparation. As an additional verification step, the author used Grok (xAI, 2026) to cross-check Claude's adversarial critiques for factual accuracy, which identified three instances where the primary AI critique attributed errors to the manuscript that were not present in the actual text. All AI outputs were independently evaluated by the author against primary sources. The integrative framework, all testable predictions, the staged experimental program, and all revision decisions are solely the author's intellectual contributions. No AI system is listed as an author, consistent with ICMJE and COPE guidelines, as AI tools cannot take responsibility for the accuracy of the work.

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

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