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Review

Nutritional Control of Intestinal Stem Cells in Homeostasis and Tumorigenesis

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Food and nutrition have a profound impact on organismal health and diseases, and tissue-specific adult stem cells play a crucial role in coordinating tissue maintenance by responding to dietary cues. Emerging evidence indicates that adult intestinal stem cells (ISCs) actively adjust their fate decisions in response to diets and nutritional states to drive intestinal adaptation. Here, we review the signaling mechanisms mediating the dietary responses imposed by caloric intake and nutritional composition (i.e., macronutrients and micronutrients), fasting-feeding patterns, diet-induced growth factors, and microbiota on ISCs and their relevance to the beginnings of intestinal tumors.

Highlights

Recent interest in dietary research and its impact on ISCs and diseases such as cancer has necessitated an integrative understanding of how diet, nutrients, and metabolism collectively regulate intestinal stemness.

To date, very little has been discussed regarding how diet regulates stem cell function beyond caloric intake and energy metabolism.

Our review focuses on the signaling mechanisms imposed by diverse dietary models.

Crosstalk between insulin/IGF-1 and ketone body signaling constitutes a key regulatory network that modulates how dietary cues and physiological factors instruct ISC fate in intestinal homeostasis and tumorigenesis.

Organismal Diet Impact on Stem Cell Fate Decisions

The intestinal epithelium, a rapidly renewing barrier of the body, has an underappreciated but emerging role as a first-line sensor of dietary nutrients. The epithelium is a dynamic single-layered structure that acts as a 'physical barrier' between luminal contents (e.g., nutrients and microbiota) and the underlying immune system, while simultaneously facilitating effective digestion and absorption (see Figure I in Box 1). In response to diverse stress stimuli from a variety of external and internal cues, constant intestinal epithelial adaptation is required. The intestinal epithelium renews itself continuously by balancing stem cell and progenitor cell self-renewal, proliferation, and differentiation to maintain homeostasis [1]. Intestinal stem cells (ISCs) nestled at the bottom of microanatomical units known as crypts actuate intestinal homeostasis (see Figure I in Box 1). ISCs generate daughter cells that are fated to differentiate and migrate up towards the crypt-villus axis and undergo functional and morphological specialization. Throughout life, intestinal tissue continually adapts to diverse extrinsic and intrinsic factors to achieve homeostasis [2].

'Organismal diet' is recognized as a powerful regulator of tissue composition by modulating stem cell self-renewal and proliferation through diet-derived nutrients and diet-induced physiology [3]. A fundamental role of dietary nutrients is to provide energy, which is calculated and presented as calories on the nutrition facts label required by the Food and Drug Administration (FDA) [4]. For decades, caloric restriction (CR, a reduction in calorie intake) has been considered to be a predominantly health-supportive dietary intervention. CR augments intestinal regeneration in response to injury by boosting the capacity of stem cells for self-renewal without an attendant increase in cancer incidence [5]. By contrast, pro-obesity diets often accelerate diseases of the intestine, such as cancer [6,7]. In addition to calories, growing evidence implicates dietary nutrients such as carbohydrates, fats, proteins, fiber, minerals, and vitamins as contributing to intestinal homeostasis independently of energy intake [8]. In addition to nutrient composition, fasting–feeding patterns and diet-induced physiology also crucially influence intestinal health and disease through endogenous metabolites as well as by hormones and growth factors [9]. These findings have provided evidence that mammalian ISCs are highly responsive to organismal nutritional status. Understanding how ISCs adjust their cell fate decisions in response to diets and

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nutritional states to maintain tissue homeostasis is at the center of the emerging field of regenerative nutrition [10].

Recent advances in intestinal stem cell biology have provided a standardized method for measuring ISC functionality, including colony-forming capacity and post-injury regeneration (Figure 1). The former is determined by the numbers of organoids (i.e., ISC colonies with mini-gut-like 3D structures and multilineage differentiation capacity) generated per crypt or per ISC in culture [11], and the latter is based on the number of proliferating or regenerating crypts per intestine in the setting of radiation-induced injury [12]. These functional assays permit investigation of how nutrients and dietary states influence ISCs *in vitro* and *in vivo*. We review below the principal dietary models, and highlight the key regulatory factors and signaling pathways that govern diet-mediated stem cell fate and their impact on intestinal homeostasis and disease.

Dietary Regulation of ISCs

CR and Fasting

Daily food consumption correlates with health and disease conditions. CR is a reduction of average daily food intake in the absence of nutrient deficiency, whereas fasting interventions primarily emphasize the duration and timing of food deprivation [13,14]. CR is defined by a 20–50% lowering of the daily amount of calories derived from carbohydrates, fats, or proteins that are usually consumed *ad libitum* [15–18]. CR is the most conserved and powerful antiaging protocol by

Box 1. The Role of ISCs in Epithelial Regeneration

Cellular Organization of the Intestine

The intestinal architecture is assembled with millions of self-renewing crypt-villus units. Villi are finger-like projections covered by a postmitotic epithelium layer that mediate the transport of absorbed nutrients (Figure IA). The base of each villus is surrounded by multiple functional invaginations, called crypts of Lieberkuhn (Figure IB) [107]. Stem cells residing in the crypts generate proliferating progenitors or transit-amplifying (TA) cells, which eventually differentiate into the major epithelial cell types that direct crypt-villus axis regeneration in the small intestine and inter-crypt regeneration in the colon [108]. TA cells undergo microenvironment-driven differentiation into an absorptive lineage or secretory lineage program (Figure IC) [5,109].

The ISC Niche

The crypt niche is a key determinant of ISC destiny. It is composed of a stromal and epithelial microenvironment. Paneth cells, enteroendocrine cells, and deep secretory are components of the ISC niche. For instance, Paneth cells produce numerous factors including WNT family member 3 (WNT3), epidermal growth factor (EGF), and NOTCH ligands that are crucial factors for stem cell maintenance and function [11,110,111]. The stromal niche consists of diverse cell types such as endothelial, neuronal, smooth muscle, and immune cells, that together with fibroblasts/myofibroblasts compose the lamina propria to support crypt function. Stromal fibroblasts provide important niche cues [112], like WNTs, EGF, R-spondins (Rspo), Noggin (NOG), Gremlin 1/2, chordin-like 1 inhibitors, and bone morphogenic protein (BMP) inhibitors, to support ISCs in homeostasis and recovery after injury [113]. Several signaling pathways are known to originate from a specific regions of the stem cell niche. For instance, WNT/β-catenin signaling is highly enriched at the crypt base to induce stem cell self-renewal and maintenance of the intestinal epithelium. BMP signaling acts in a paracrine fashion to dampen stem cell self-renewal, and intestinal niche components counteract BMP signaling by producing BMP antagonists to promote stemness (Figure IC) [114,115]. Finally, extracellular matrix, immune cells, and cytokines also contribute to the intestinal niche [112]. Therefore, a combination of epithelial, mesenchymal, and immune cell-derived cues help to instruct ISC fate decisions.

ISCs

Active ISCs are subgrouped according to their specific locations in the crypt base – crypt base columnar (CBC) cells and DNA label-retaining cells (LRCs), also known as 'position 4,' or '+4', that reside directly above the Paneth cells (Figure IA,B) [116]. CBCs are continuously cycling cells that are intercalated between Paneth cells at the small intestinal crypt base, whereas, in the colon crypt, ISCs are located between Reg4-positive deep crypt secretory (DCS) cells that function as an epithelial niche akin to Paneth cells in the small intestine [112,117]. In a key 2007 manuscript, Barker *et al.* discovered that CBCs express high levels of leucine-rich repeats containing G protein-coupled receptor 5 (Lgr5) [118]. These Lgr5-expressing ($Lgr5^+$) ISCs produced daughter ISCs and all mature cell types of the intestine [107].

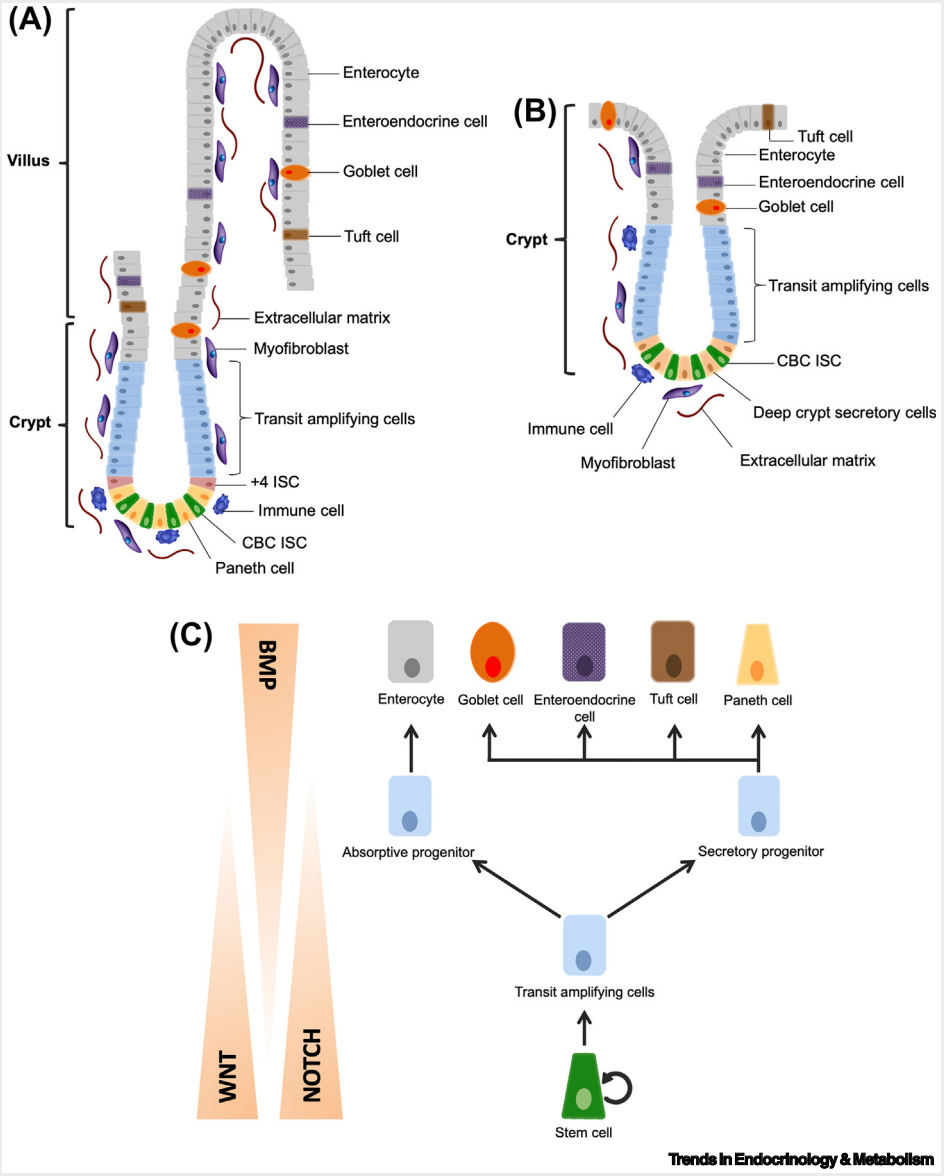


Figure I. Structural Organization of the 'ISC Niche'. Schematic illustration of niche composition in the (A) small intestine and (B) colon. (C) Hierarchical model of ISC lineages and differentiation. In the mammalian intestine, the epithelium is organized into crypt-villus units. Current nomenclature describes two stem cell populations: CBC cells that are localized between Paneth cells at the crypt base, and +4 stem cells located above the Paneth cells. The colonic epithelium niche does not contain Paneth cells, and ISCs are located between deep crypt secretory cells that have a niche function. Stem cells give rise to TA cells that differentiate into nutrient-absorbing enterocytes or mucus-secreting goblet cells, hormone-producing enteroendocrine cells, and niche factor-secreting cells called Paneth cells and tuft cells. Niche factors arise from the subepithelial mesenchyme, and mostly from stromal fibroblasts/myofibroblasts. Extracellular matrix, immune cells, and cytokines also contribute to the intestinal niche. Intestinal homeostasis is controlled by various signaling pathways that regulate the balance between stem cell self-renewal and differentiation. For instance, WNT signaling is crucial for stem cell proliferation, whereas BMP signals inhibit self-renewal and promote differentiation by deactivating WNT/β-catenin signaling. Lastly, NOTCH signaling targets the stem cell population to maintain proliferation and promote their survival.

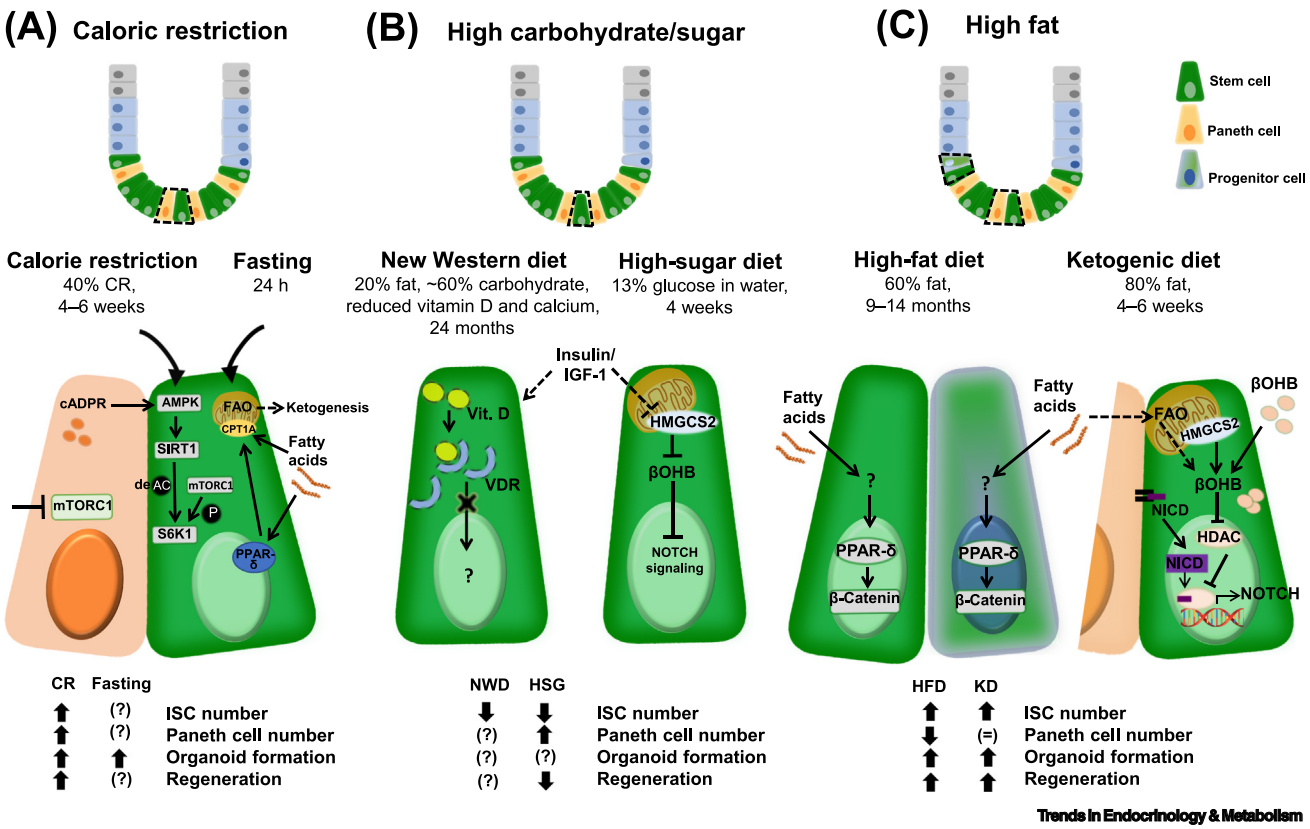


Figure 1. Nutritional Regulation of Intestinal Stem Cell (ISC) Function and Fate. (A) Impact of caloric restriction (CR) on ISCs. CR (40% CR, for 4–6 weeks) amplifies ISC and neighboring niche cell numbers and enhances stem cell self-renewal capacity in response to reduced mammalian target of rapamycin (mTOR) signaling from Paneth cells. CR boosts the expression of bone stromal antigen 1 (Bst-1) for the generation of cyclic ADP-ribose (cADPR) which enters ISCs to promote self-renewal in a sirtuin 1 (SIRT1)-dependent manner. Fasting (24 h), another CR regimen, induces the cellular function of ISCs and progenitor cells by promoting fatty acid oxidation (FAO) through activation of a peroxisome proliferator-activated receptor (PPAR)-δ program. Abbreviations: deAC, deacetylation; P, phosphorylation. (B) Effects of high-carbohydrate/sugar-containing diets on ISCs. New Western diet (NWD; 20% fat, ~12% protein, ~60% carbohydrate, reduced calcium, vitamin D (Vit. D), and fiber with reduced levels of methyl donor nutrients, for 24 months) decreases ISC numbers and their self-renewal capacity through reduced levels of vitamin D and calcium. High-sugar diet (HSD; 13% glucose in drinking water *ad libitum* for 4 weeks) decreases ISC number by diminishing HMGCS2 expression and β-hydroxybutyrate (βOHB) levels. Glucose-mediated suppression of intestinal ketogenesis shows that NOTCH activity correlates with βOHB concentrations. (C) Impact of high-fat diet (HFD) on ISCs. HFD consumption induces cell number and self-renewal function through triggering PPAR-δ signaling in both stem cells and progenitors. This diet enables ISCs to become niche-independent and relates to the increased tumorigenic properties of the stem cell niche. Ketogenic diet (KD) elevates ISC number and function using βOHB-mediated class I histone deacetylases (HDAC) inhibition that reinforces the NOTCH signaling in stem cells. Key: ↑, increased; ↓, decreased; =, equal (unchanged).

improving life quality and health-related parameters, such as decreased insulin and IGF-1 levels, in CR-fed mice as well as in other diverse species [16,19,20]. Typically, intermittent fasting, also known as alternate day fasting (ADF), is a dietary regimen in which food is only available every other day. However, owing to the lack of terminology standardization, different fasting regimens are used as intermittent fasting models. For example, the 5:2 diet – unrestricted eating for 5 days of the week and 2 days of restricted caloric intake – is sometimes referred to as intermittent fasting [21]. Both CR and fasting decrease insulin and IGF-1 levels and improve life quality [21–23].

It is well established that the mammalian intestine responds to dietary habits and fluctuations. Such dietary changes can be leveraged to promote disease prevention and/or restoration of intestinal health. CR acts in part by boosting the function of tissue-specific adult stem cells, and specifically enhances stem cell availability and activity without increasing the risk of cancer

incidence [24]. Recent studies illustrate that 30–40% CR diets for more than 2 weeks reduce small intestinal mass and lead to morphological changes in villi and crypts (e.g., shorter villus length and larger crypt size) *in-vivo* [17,25].

CR (40% restriction for 4–28 weeks) amplifies the numbers and proliferation rates of $Lgr5^+$ ISCs at the expense of more differentiated cells in response to reduced mammalian target of rapamycin (mTOR, a multiprotein complex master sensor of growth factors and amino acids) signaling from neighboring Paneth cells. The decreased number of mature enterocytes indicates that CR promotes ISC self-renewal. Mechanistically, downregulation of mTOR complex 1 (mTORC1) induces the expression of bone stromal antigen 1 (Bst-1), an enzyme that converts NAD^+ to paracrine factor cyclic ADP-ribose (cADPR), and which drives ISC self-renewal and expansion in the intestinal crypt (Figure 1A) [17]. CR also promotes the regeneration capacity of ISCs: crypts from CR mice form greater numbers of organoids than those from *ad libitum* mice, and CR augments ISC regeneration and the number of surviving crypts after irradiation-induced damage [17].

Building on these early findings, another study demonstrated that CR Paneth cells secrete cADPR to promote SIRT1 activity and proliferation of ISCs in an mTORC1-dependent manner (Figure 1A) [25]. Moreover, inhibition of SIRT1 activity leads to inactivation of mTORC1, which dampens the effect of CR on ISC activity (Figure 1A) [26]. CR can also modulate the regenerative capacity of the intestinal epithelium through effects on 'injury-resistant reserve' ISCs (12 Gy of irradiation) by inhibiting mTORC1 signaling in this population to protect them from radiation-induced injury [26].

These findings demonstrate a crucial role of mTOR signaling in linking stem cell function to dietary status. Fasting interventions can also modulate ISC function after injury or during aging [24]. Short-term 24 h fasting in the small intestine promotes ISC and progenitor cell function in young and aged mice, including robustly boosting their organoid-formation capacity. In a clinical setting, a 4 day fasting-mimicking diet (FMD) (50% of standard daily calorie intake on day 1, and 10% of standard daily calorie intake on days 2–4) shows promise in alleviating inflammatory bowel disease (IBD)-associated inflammation by modulating ISC activity [27]. Mechanistically, fasting triggers ISCs to switch from carbohydrate-based metabolism towards fatty acid metabolism. Short-term fasting promotes fatty acid oxidation (FAO) in ISCs by liberating free fatty acids (FFAs) from adipose stores, which stimulate a peroxisome proliferator-activated receptor (PPAR)-δ program (Figure 1A) [28]. Interestingly, the dependency to FAO varies along the length of the small intestine, and this is regulated by PR domain-containing 16 (Prdm1). Loss of the Prdm16-mediated FAO particularly perturbs the survival and functions of ISCs in the upper intestine [29]. A recent study also finds that FAO supports ISC self-renewal via HNF4-mediated lipid sensing [30].

Western-Style Diet (WD)

The WD arose from historical changes in diet and lifestyle following the industrial revolution in the Western world. It typically includes refined grains, processed foods, sweetened beverages, and high-fat dairy products. This dietary style alters normal physiological levels of hormones (such as increased insulin and IGF-1), circulating metabolites, and increases the risk of insulin resistance and colorectal cancer (CRC) [31–34]. In animal experiments, a WD has been operationally defined as a diet with a relatively high fat (~35%) and high carbohydrate (~49%) content [35]. In 2001, a new WD (NWD) with reduced calcium, vitamin D, fiber, and levels of methyl donor nutrients (e.g., folic acid, methionine, choline, and vitamin B12) was developed as a revised WD to mimic the low intakes of fruit and vegetables based on the dietary habits of Western countries [36,37]. NWD consumption causes crypt hyperproliferation and dysplasia, leading to dysplasia and adenoma formation [36]. In addition, NWD diminishes $Lgr5^+$ stem cell self-renewal in a vitamin D-dependent manner (Figure 1B) [38]. The vitamin D receptor (VDR) gene is expressed

in the Lgr5$^+$ stem cell signature gene set, and VDR expression decreases as Lgr5 stem cells differentiate into specialized cells of the villi [39]. Lastly, inactivation of VDR in Lgr5$^+$ stem cells hampers their activity, indicating that vitamin D is an important nutrient for the maintenance of stemness and tissue homeostasis [38–40].

High-Sugar Diet

The increased consumption of high-sugar diets leads to obesity, diabetes, and cancer [41]. High-sugar foods or drinks containing 60–70% of fructose, glucose, or sucrose elevate blood glucose and insulin levels, which are the root cause of the metabolic disorders and diseases [41–43]. In *Drosophila melanogaster*, a high-sugar diet (1 M sucrose versus control 0.15 M sucrose) induces oxidative stress, which upregulates the stress signaling Jun-N-terminal Kinase (JNK) pathway and consequently downregulates the proregenerative signaling Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway to promote ISC differentiation [44,45]. In mice, standard chow diet with glucose-supplemented drinking water (13% glucose, for 4 weeks) diminishes the number and function of ISCs and promotes their differentiation towards Paneth and goblet cells. Furthermore, glucose supplementation also dampens the ability of ISCs to regenerate the intestine after radiation-induced injury. Mechanistically, glucose dampens stemness through glucose- and insulin-mediated suppression of intestinal ketogenesis, a metabolic process that is crucial for stem cell signaling via NOTCH (Figure 1) (discussed further in the Ketogenic Diet section) [38]. In contrast to the observations *in vivo*, glucose treatment *in vitro* stimulates glycolysis and activates the nutrient-sensing AKT/HIF-1α signaling pathway to induce proliferation of murine intestinal crypt cells [39]. The lack of diet-induced physiology (e.g., insulin and IGF-1 levels) and gut microbiota in the culture system may partially explain this disassociation between the *in vitro* and *in vivo* effects of glucose on ISC energy metabolism and cell fate decisions.

High-Fat Diet (HFD)

Excess high-fat food intake is recognized to play an important role in the development of obesity and multiple life-threatening diseases such as cardiovascular disease, type 2 diabetes, hypercholesterolemia, metabolic syndrome, and cancer in humans. Pro-obesity HFDs typically contain about 32–60% of calories from fat and produce alterations in the levels of circulating plasma metabolites, hormones (insulin, adiponectin), and lipids (such as triglycerides, FFAs, and cholesterol) [41,46]. In mice, a pro-obesity HFD crucially regulates the intestinal homeostasis and increases tumor incidence (discussed in the section on the Connection Between Diet, ISCs, and Cancer) [6,47–49]. Long-term HFD (60% fat diet, 9–14 months) elevates the Lgr5$^+$ ISC numbers and self-renewal that coincides with a decrease in Paneth cell numbers [6]. These HFD effects on promoting ISC self-renewal eventually lead to a reduction in villus length, a decline in villous enterocyte numbers, and an increase in crypt depth. HFD consumption also alters ISC function and niche-dependence (Box 1). *In vitro*, HFD-derived ISCs (without coculturing with Paneth cells) have an increased capacity to initiate organoids compared to control ISCs, suggesting that HFD induces autonomous changes in ISCs and allows Lgr5$^+$ ISCs to depend less upon intestinal niche factors (Box 1) Another study reports that a pro-obesity HFD (45% fat diet, 20 weeks) causes ISCs to rely upon systemic insulin/IGF-1 signaling. *In vivo*, a HFD induces hyperinsulinemia and augments ISC numbers and proliferation, while decreasing Paneth and goblet cell numbers. *In vitro,* isolated ISCs from HFD-fed mice require exogenous insulin or IGF-1 treatment to maintain organoid-forming capacity [47]. Moreover, intestinal crypts in HFD-fed mice display enhanced regenerative capacity after challenge by radiation-induced injury *in vitro* and *in vivo* [6]. Together, these data suggest that a HFD boost the numbers and regenerative capacity of ISCs.

Mechanistically, HFD induces PPAR-δ, a ligand-activated transcription factor that modulates the metabolic responses and self-renewal of ISCs and niche-based progenitors [6]. Crypt-derived

organoids treated with fatty acid constituents (such as palmitic acid, oleic acid, or a lipid mixture) display enhanced self-renewal capacity in a PPAR-δ-dependent manner [6]. Pharmacological activation of PPAR-δ augments the proliferation of stem cells and progenitors, indicating that enforced PPAR-δ signaling mimics aspects of the HFD in the mammalian small intestine (Figure 1C) [6].

In the colon, one study describes that a HFD (60% fat diet, 12 weeks) elevates $Lgr5^+$ ISC number and function by decreasing adiponectin-mediated apoptosis. In organoids from lean mice, agonist-activated adiponectin receptor signaling causes a decrease in $Lgr5^+$ stem cell number and an increase in apoptosis. However, this adiponectin signaling-induced apoptosis was diminished in organoid cultures from obese mice, indicating that colonic $Lgr5^+$ stem cells have impaired adiponectin signaling. Because obesity development is followed by the expansion of adipose tissue and impaired adipocyte function in maintaining energy homeostasis, this finding may explain how stem cell accumulation takes place in obesity and how these changes can be targeted therapeutically [48].

Ketogenic Diet (KD)

The KD is designed to induce a fasting-like metabolic process that converts fatty acids and amino acids into ketone bodies (i.e., ketogenesis). Early physicians introduced it as a high-fat (>65%), adequate-protein (~15%), and low-carbohydrate (<10%) diet for treating epilepsy in the 1920s [50]. KD recapitulates some metabolic aspects of dietary restriction and fasting such as reduced insulin levels, dependence on fatty acid instead of glucose metabolism, and production of ketone body molecules such as β-hydroxybutyrate (βOHB) and acetoacetate [19]. Therefore, KD has also been used as a dietary intervention for glucose metabolism-related disorders and diseases [43]. In fact, in the past decade, KD has gained recognition as a new therapeutic application to prevent or treat age-related diseases including cancers [51–53]. However, the mechanistic role of KD in intestinal stem cell and tissue regeneration has only started to be uncovered [54].

A recent study reports that ISCs express significantly higher amounts of the enzyme 3-hydroxy-3-methylglutaryl-CoA synthetase 2 (HMGCS2), a rate-limiting step in ketone body production, and contain greater levels of the ketone body molecule βOHB compared to their more differentiated progeny. Deletion of *Hmgcs2* depletes intracellular βOHB in ISCs, leading to premature differentiation towards the secretory lineage and eventually resulting in loss of ISC homeostasis. Loss of *Hmgcs2* also impairs the regenerative capacity of ISCs after radiation injury. The defects in *Hmgcs2*-null ISCs are restored upon administration of oral βOHB administration [44], highlighting a role for ketone bodies in ISC maintenance. HMGCS2-dependent stemness is mediated by βOHB inhibition of histone deacetylases (HDACs). One pathway that is activated and reinforced by this process is the NOTCH program, a signaling pathway that controls the cell fate decisions of ISCs [55]. Notably, a KD (80% fat, 5% carbohydrate, 15% protein diet for 4–6 weeks), by elevating βOHB and NOTCH activity, boosts ISC numbers and enhances ISC function as assessed in organoid assays and in post-injury regeneration (Figure 1C) [54]. In contrast to the definitive KD, a ketone-supplemented diet (rodent chow supplemented with 1,3-butanediol ketone diesters) induces intestinal cell differentiation accompanied by an increase in enterocyte, goblet, and Paneth cell populations [56]. It is possible that excessive ketone bodies or chemical byproducts of ketone diesters may induce the stress responses that promote intestinal differentiation [57].

Impact of the Microbiota on ISCs

The intestine hosts a diverse intestinal microbial community, or 'microbiota', that is composed of a variety of microorganisms such as bacteria, fungi, and viruses [58,59]. The gut microbiota influences intestinal architecture, such as villus structure and crypt depth. For instance, the villi

of germfree mice are longer and thinner in the distal small intestine. In addition, the crypts are shallower and have fewer proliferating ISCs than conventional (microbiota-competent) mice [58].

Gut microbiota species (such as *Lactobacillus* and *Bifidobacterium)* regulate ISC self-renewal and differentiation decisions to maintain intestinal homeostasis and respond to bacterial infections [58,59]. In the fly midgut, pathogen- and bacterial infection-based damage boosts cytokine secretion (e.g., unpaired family, Upd, Upd2, and Upd3) to activate the JAK–STAT signaling pathway that drives ISC proliferation [60]. Commensal gut bacteria benefit the host in different ways, including aiding digestion, building the immune system, competing with harmful microbes, and, importantly, stimulating ISC activity. For instance, *Lactobacillus,* a beneficial commensal bacteria in humans, triggers intestinal enterocytes to produce NADPH oxidase-dependent reactive oxygen species (ROS) and subsequently promote JNK signaling to increase the number of ISCs [61,62]. The symbiont-generated lactate appears to be an important regulator of $Lgr5^+$ ISC-mediated epithelial homeostasis [63]. In mice, administration of lactic acid-producing bacteria such as *Bifidobacterium* and *Lactobacillus* enhances the numbers of ISCs, Paneth cells, and goblet cells. Symbiont-derived lactate interacts with G protein-coupled receptor 81 (GPR81) on Paneth and stromal cells to elicit ISC proliferation through WNT/β-catenin signaling. *Lactobacillus* spp. lacking the activity of lactate dehydrogenase (deficient in lactate production) fail to promote ISC-driven regeneration. Thus, symbiont-derived lactic acid plays a crucial role in maintaining intestinal homeostasis in a GPR81-dependent manner [63].

The epithelium engages with resident luminal bacteria by recognizing bacterial components through pattern recognition receptors (PRRs) such as membrane-bound Toll-like or cytoplasmic Nod-like receptors (TLRs/NLRs). TLR signaling modulates intestinal homeostasis by impacting on crypt-based proliferation and apoptosis. For instance, TLR4 activation of $Lgr5^+$ stem cells controls their proliferation by suppressing WNT and NOTCH signaling [59,64] and biases ISC differentiation towards goblet cells [65]. Thus, there is a complex regulatory network between stem cell niche and intestinal microbial composition which impinges on nutrient absorption, endocrine signaling, and immune response.

Microbiota composition has been shown to respond to dietary modifications. CR is associated with significant enrichment of protective bacteria such as *Lactobacillus* spp. in both humans and mice [66]. In CR mice, *Lactobacillus* spp. and Lachnospiraceae contribute to the protection of the intestinal barrier from inflammation-related damage and improve gut barrier function [67]. CR significantly impacts on intestinal flora, leading to a prominent increase of *Lactobacillus rhamnosus* GG, and has protective effects on intestinal radio-/chemotoxicity by preserving the proliferative capacity of crypt cells and ISC function (Figure 2) [68]. Species of the Lachnospiraceae family are also enriched by CR and can mitigate the side effects of chemotherapy by increasing ISC numbers (Figure 2) [67]. In addition, a fasting-mimicking diet (FMD) elevates microbial populations of Lactobacillaceae and Bifidobacteriaceae in an inflammatory bowel disease (IBD) mouse model, and alleviates disease symptoms by enhancing intestinal regeneration (Figure 2) [27].

There is mounting evidence that a KD alters gut microbial diversity and composition. These changes lead to decreased microbial heterogeneity and selection for beneficial bacteria (e.g., *Akkermansia muciniphila* and *Lactobacillus* spp.) as a result of reduced carbohydrate intake [69]. A recent study further indicates that a KD modifies the gut microbiota in a manner distinct from a HFD, owing in part to the production of ketone bodies in the host, and raising the possibility of diet-induced changes in host microbiota composition (Figure 2) [70]. Although the underlying mechanism remains unclear, a high-sugar diet is associated with a decreased diversity of

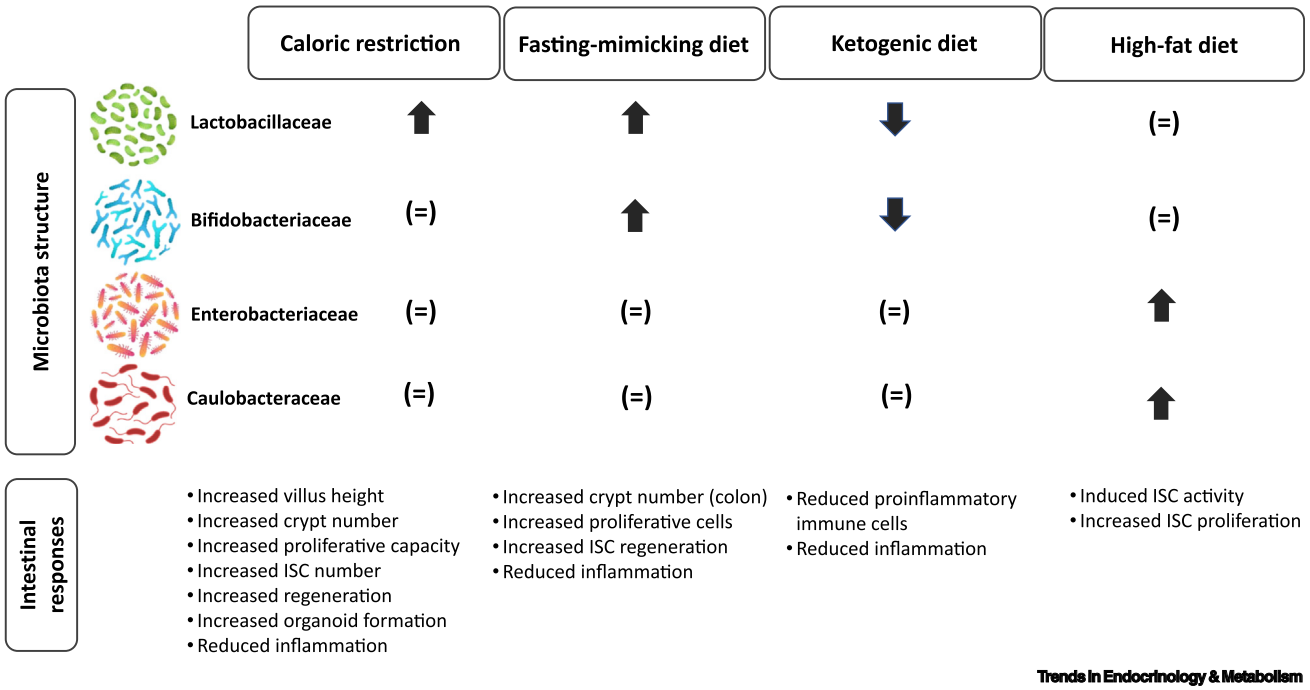


Figure 2. Impact of Diet-Induced Alterations in Gut Microbiota on Intestinal Responses. Dietary interventions affect the structure of intestinal microbiota, which in turn alters the intestinal architecture, inflammatory responses, and intestinal stem cell (ISC) activity. For instance, caloric restriction (CR) enriches protective bacteria of the Lactobacillaceae and Bifidobacteriaceae families, and this increases villi height and crypt numbers by promoting the proliferation of crypt cells. It also suppresses inflammatory responses by enhancing intestinal regeneration. Similar changes are found in the mice fed on a fasting-mimicking diet. On the other hand, a ketogenic diet decreases Lactobacillaceae and Bifidobacteriaceae abundance while diminishing the inflammatory responses by reducing the proinflammatory immune cells in the small intestine. Unlike the ketogenic diet, high-fat diet alters intestinal microbiota by enriching for Enterobacteriaceae and Caulobacteriaceae, and this stimulates the proliferation and regenerative activity of ISCs. Key: ↑, increased; ↓, decreased; =, equal (unchanged).

commensal gut bacteria and leads to obesity and metabolic syndrome in a *Drosophila*-based study [44]. Interestingly, HFD markedly alters both the abundance and composition of microbiota, especially by enriching for species of the orders Enterobacterales and Caulobacterales. This eventually elicits major effects on intestinal structure and function by triggering the activation of ISCs (Figure 2D) [71]. Overall, diet-derived nutrients and physiology converge to regulate microbial populations, and these consequently influence gut homeostasis.

The Connection Between Diet, ISCs, and Cancer

It is generally assumed that the 'cells-of-origin' of diverse types of cancer are tissue-specific adult stem cells. Given that adult stem cells are long-lived, it is believed that they accumulate the initial mutations that drive tumorigenesis. These mutant adult stem cells either directly or indirectly through their progeny engender frank cancers. For example, in the intestine, genetic loss of function of the adenomatous polyposis coli (*APC*) gene, a mutation that commonly occurs in CRC, in ISCs leads to the rapid and lethal generation of adenomas, whereas *APC* loss in non-stem cells is far less tumorigenic [72,73]. Therefore, dietary models that promote ISC activity may increase cancer incidence by enlarging the pool of cancer-initiating stem cells (i.e., ISCs) [74,75].

CR and fasting have shown promising cytotoxic effects on established tumors by targeting the vulnerability of cancer cells to nutrient deficiency and reduced nutrient-sensing signaling [16,76,77]. CR, by reducing circulating insulin/IGF-1 levels, dampens phosphoinositide 3-

kinase (PI3K)/protein kinase B (AKT) signaling pathways and suppresses cell survival in a human cancer cell line (SW620) derived from colon, accompanied by increased expression levels of Forkhead box O (FOXO) target genes [16,76,77]. CR also inhibits colon tumor cell (MC38) growth through modulation of hormone-stimulated nuclear factor κB (NF-κB) activation and inflammation-related gene expression (Figure 3A) [78–80]. CR sensitizes tumors to chemotherapy by driving a metabolic shift from glycolysis to oxidative metabolism in CRC cells. Specifically, overproduction of ROS and decreased levels of ATP trigger the initiation of apoptosis in cancer cells [81]. Similarly, by exploiting the vulnerability of cancer cells to nutrient deprivation, fasting interventions have demonstrated promising therapeutic potentials in treating a variety of cancers [82], including the hard-to-treat human (HCT116, DLD-1) and murine (CT26) KRAS-mutant CRC cells in combination with vitamin C treatments [83]. However, the underlying

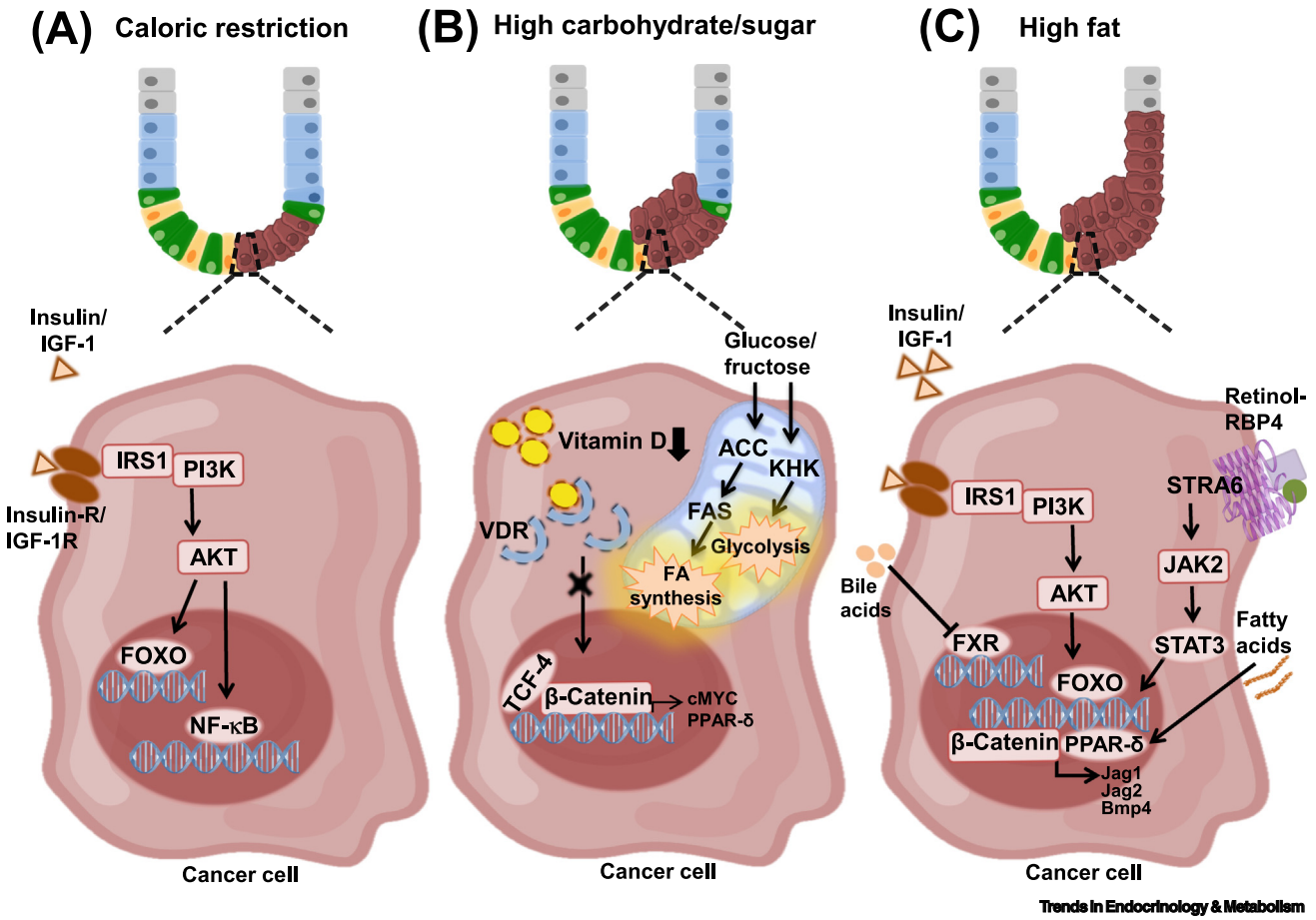


Figure 3. Dietary Regulation of Intestinal Tumorigenesis. (A) Impact of caloric restriction (CR): CR decreases insulin/IGF-1 signaling and increases the expression of Forkhead box O (FOXO) target genes through the modulation of phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) signaling pathways. In addition, CR can inhibit colon tumor cell growth by modulating hormone-stimulated nuclear factor-κB (NF-κB) activation. (B) Impact of high-carbohydrate/sugar diet: decreased activation of VDR can induce the expression of β-catenin target genes *MYC* and *PPARD*, and this may boost tumor-formation capacity. With the increased levels of glucose and fructose, ketohexokinase (KHK) consumes fructose and induces glycolysis, which serves as a carbon source of fatty acid (FA) synthesis. Increased expression of lipogenic enzymes including acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) supports fatty acid synthesis. (C) Impact of pro-obesity high-fat diet (HFD): activation of insulin/IGF-1 signaling triggers AKT (protein kinase B, PKB) and phosphorylation of FOXO factors which leads to their exclusion from the nucleus. Abundant dietary fatty acids activate peroxisome proliferator-activated receptor δ (PPAR-δ) signaling and the transcription of β-catenin targets. HFD can also induce the circulating retinol-binding protein 4 (RBP4–STRA6) pathway signaling, which leads to the activation of Janus kinase/signal transducer and activator of transcription (JAK–STAT). Bile acids that are increased by a HFD antagonize intestinal farnesoid X receptor (FXR) function and induce DNA damage and proliferation in $Lgr5^{+}$ cancer stem cells.

mechanisms of how CR and fasting differentially regulate normal and malignant ISCs remain unclear.

In contrast to CR, HFD activates the progrowth MAPK/ERK and PI3K/AKT/mTOR signaling pathways, and induces tumor growth and progression in a xenograft model of colon cancer [84]. Similarly to CR, HFD expands the Lgr5^{+} ISC pool. More importantly, a HFD endows organoid- and tumor-forming capacity to non-ISC progenitors, a feature normally ascribed to ISCs (Figure 1C) [6]. Mechanistically, HFD-derived fatty acids stimulate a PPAR-δ transcriptional program that includes fat metabolism and WNT/β-catenin signaling in both ISCs and progenitors (Figure 3C) [6,85]. Recent studies illustrate that, in stem cells of the intestine and the hair follicle, loss of mitochondrial pyruvate carrier 1 (Mpc1) restricts the participation of pyruvate in the tricarboxylic acid (TCA) cycle and compensatorily induces FAO, which subsequently promotes stemness and leads to a protumorigenic phenotype that shares the common feature of HFD-activated PPAR signaling [86–88]. It is also possible that a HFD promotes tumorigenesis through lysophosphatidylcholine acyltransferase 3 (Lpcat3)-mediated dietary-lipid absorption, phospholipid remodeling, and cholesterol homeostasis, because disruption of *Lpcat3* stimulates ISC proliferation and significantly enhances tumor formation in *Apc*^{min} mice [89,90]. In addition to fatty acids, bile acids (BAs) are elevated in mice fed a HFD and are a recognized risk factor for CRC. In normal Lgr5^{+} stem cells, BAs are the endogenous agonists of G protein-coupled bile acid receptor 1 (GPBAR1/TGR5) that augments Yes-associated protein 1 (YAP1) signaling. The BA–TGR5 axis increases stem cell number, promotes proliferation, and enhances the organoid-forming and tissue-regenerating functions of ISCs [91]. BAs, such as tauro-β-muricholic acid (T-bMCA) and deoxycholic acid (DCA), also antagonize intestinal farnesoid X receptor (FXR, the master regulator of BA homeostasis) signaling and provide a growth advantage to Lgr5^{+} ISCs via WNT signaling [92] to control crypt villus structure and gut homeostasis (Box 1). In the *Apc*$^{min/+}$ tumor mouse model, increased levels of BAs in a HFD repress FXR signaling and enhance the proliferation and DNA damage of Lgr5^{+} ISCs, promoting tumor progression (Figure 3C) [92]. Conversely, pharmacological activation of intestinal FXR by its agonist (FexD) dampens HFD-induced intestinal cancer progression by restraining Lgr5^{+} cell growth and skewing differentiation towards goblet cells [92]. A recent study additionally reports that elevated vitamin A-bound serum retinol-binding protein 4 (RBP4) and retinoic acid 6 (STRA6), which are implicated in colon stem cell self-renewal, may also contribute to HFD-induced tumorigenesis (Figure 3C) [93]. Knockdown of RBP4 or STRA6 in colon cancer cells decreases NANOG and SOX2 levels, two key regulators of stem cells, as well as reducing tumor initiation in a xenograft mouse model [93,94]. Mechanistically, dietary fats stimulate RBP4/STRA6 signaling in colon cancer xenografts to activate the JAK–STAT3 pathway and boost Lgr5 expression and the tumorigenicity of ISCs [93]. In summary, all these studies on how a pro-obesity HFD impacts on ISCs indicate that this dietary regimen engages many diverse pathways in ISCs that collectively contribute to intestinal tumorigenesis.

In addition to carbohydrate-induced insulin/IGF-1 signaling, a NWD that contains low dietary vitamin D3 levels has protumorigenic effects through diminished VDR signaling in Lgr5^{+} ISCs [38]. A recent study suggests that vitamin D3 and calcium supplementation of the diet of mice quelled the tumorigenic capacity of the NWD, as does VDR loss in ISCs [38]. VDR functions as a tumor suppressor by inhibiting the expression of the oncogenic WNT/β-catenin targets *MYC* and *PPARD* (Figure 3B) [95]. Low levels of vitamin D3 in a NWD induce WNT activation by dampening VDR signaling in reserve ISCs (Bmi-1^{+} cells) and tumor development [96]. It is, however, unclear whether vitamin D supplementation has preventative effects against colon cancer in humans [97].

Sugar and corn syrup consumption, for example in beverages, is a recently recognized risk for CRC that acts independently of obesity. High-fructose corn syrup (HFCS), a sweetener composed of both fructose and glucose, is commonly used to sweeten processed foods and soft drinks. In mice, daily oral consumption of HFCS (25% HFCS in drinking water for 9 weeks) significantly augments tumor size and tumor grade [98]. High fructose and glucose concentrations in the intestinal lumen lead to the generation of fructose-1-phosphate via ketohexokinase (KHK, also known as fructokinase), a key enzyme in fructose metabolism, and also stimulate glycolysis [98] (Figure 3B). HFCS treatment also augments the expression of lipogenic enzymes, including acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS),

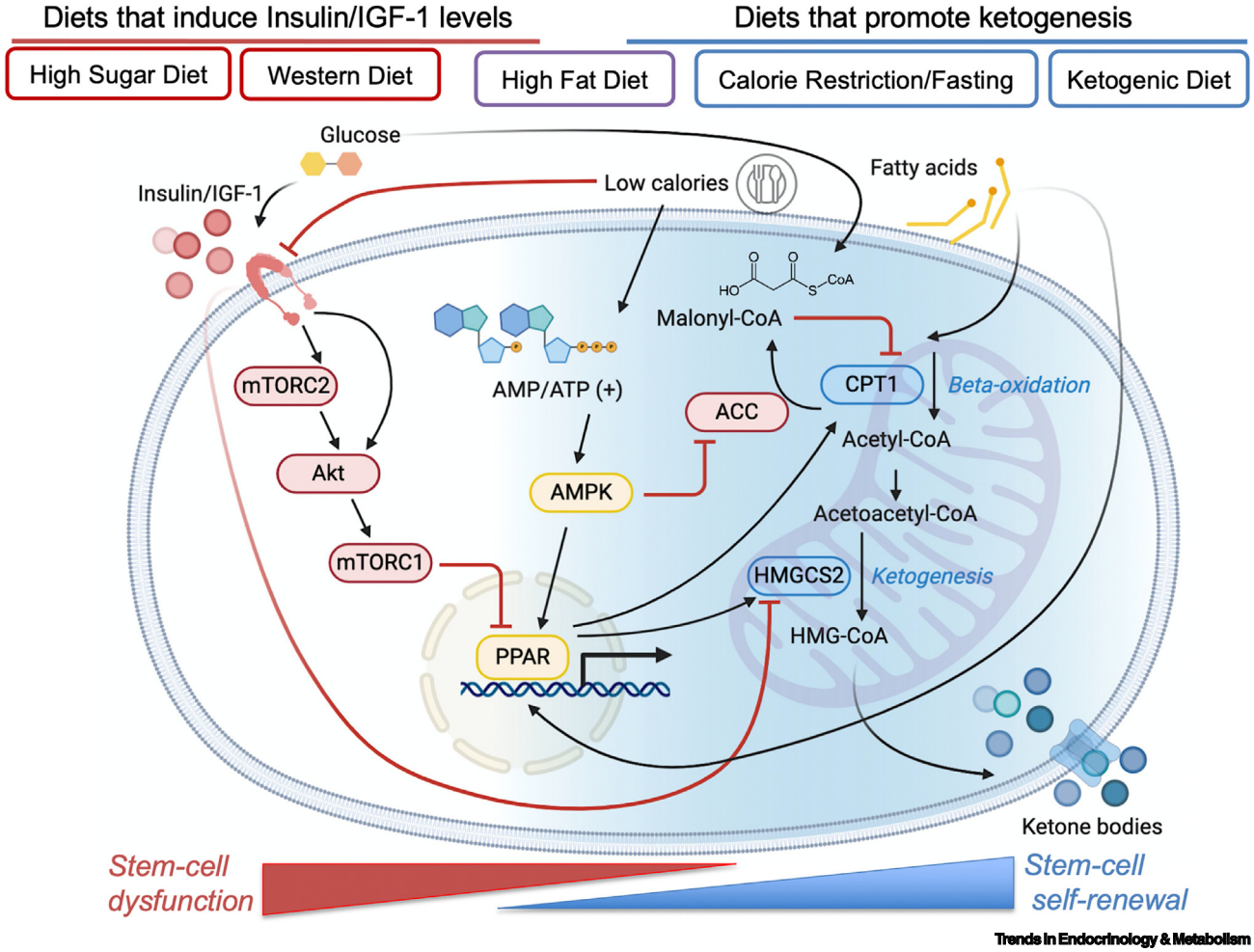


Figure 4. Crosstalk between Insulin/IGF-1 and Ketone Body Signaling, and the Integration of Nutritional Signaling Pathways Regulating Intestinal Stem Cell (ISC) Fate Decisions. Caloric restriction (CR), fasting, and the ketogenic diets increase the levels of free fatty acids that activate peroxisome proliferator-activated receptors (PPARs), promote β-oxidation (FAO) through carnitine palmitoyltransferase 1 (CPT1), and generate ketone bodies through 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2), eventually inducing ISC self-renewal. For CR and fasting, energy deficiency (low calories) also increases the AMP/ATP ratio, which activates AMP-activated protein kinase (AMPK) and activates PPARs to promote FAO and ketogenesis. AMPK also enhances β-oxidation by inhibiting acetyl-CoA carboxylase (ACC) activity, which otherwise catalyzes the conversion of acetyl-CoA to malonyl-CoA and negatively regulates FAO. A high-fat diet (HFD) induces both insulin/IGF-1 and ketone body production, leading to unpredictable ISC phenotypes. High-carbohydrate-based dietary models such as the high-sugar diet and the Western diet diminish the self-renewal capacity of ISCs, possibly by suppressing ketogenesis and FAO through increased glucose and insulin/IGF-1 levels. Activated insulin/IGF-1 signaling by increased glucose levels directly or indirectly (through the inhibition of PPARs) inhibits HMGCS2. Upon activation of insulin/IGF-1 signaling, mammalian target of rapamycin complex 2 (mTORC2) and AKT are activated and induce mTORC1 that eventually inhibits PPARs. In addition, intracellular glucose can directly inhibit FAO by malonyl-CoA-mediated inhibition of CPT1.

and drives fatty acid synthesis – a metabolic phenotype that favors highly proliferative cancer cells [98].

Concluding Remarks

An overarching goal of dietary research is to identify the nutritional factors that regulate tissue homeostasis and to provide dietary guidelines for health promotion or disease prevention. As reviewed in the previous text, diet-derived nutrients and their metabolite intermediates play an important role in instructing ISC self-renewal, differentiation, and lineage decisions by activating a variety of signaling cascades that influence tissue homeostasis (Figure 1) and tumor formation (Figure 3). Beyond the aspects of calories and cellular energy, these studies reveal a new category of regulatory networks by which ISCs integrate and translate the nutritional messages into specific cell fate decisions. Interestingly, crosstalk between insulin/IGF-1 and ketone body signaling emerges to be the hub that integrates the nutritional signaling pathways to control ISC fates (Figure 4). In this integrative view, dietary models that increase fatty acid utilization, such as a KD and fasting, promote ISC self-renewal through ketone body-mediated cell signaling or metabolism [28,54]. By contrast, diets that promote sugar/glucose metabolism, such as a high-carbohydrate WD [32–34] and a high-sugar diet [42,43,98], hamper stemness and perturb differentiation, potentially by suppressing ketone body production through glucose metabolism [99,100] and insulin signaling (Figure 4). Intriguingly, a pro-obesity HFD induces insulin and paradoxically drives ketone body production (a process normally inhibited by insulin) to promote ISC self-renewal, secretory lineage differentiation, and tumorigenesis [49,101]. Coordinated stimulation by insulin and ketone bodies may underlie the tumorigenic propensity of particular diets.

There are several issues to consider for future dietary studies aimed at deciphering the nutritional control of adult stem fate (see Outstanding Questions). First, the formulations of dietary models are not precisely defined. This ambiguity makes it difficult to draw conclusions because minor differences in nutritional formulations can often exert distinct effects on endocrine and metabolic responses [70]. For instance, similarly to an isocaloric KD, HFD typically contains more than 40% of fat [6,47–49]. However, unlike KD, HFD contains ~15% more carbohydrates and extra calories depending on food intake [6,47–49], which favors insulin production and insulin/IGF-1 signaling at the beginning (<6 weeks) [47,102] and delays the onset of ketone body production (Figure 4). Second, even with identical dietary formulations, the effect of a chronic diet varies with duration and adaptive dietary responses. In the case of chronic HFD (>3 months), the shift towards ketone production becomes more evident after the development of HFD-induced insulin resistance [101]. On the other hand, a less pronounced increase of ketone body levels is found for a long-term KD (>3 months) because of a secondary metabolic adaptation to utilize ketone bodies as a fuel [103]. Third, the adaptation to a diet involves multiorgan coordination, and this is often not systemically addressed. CR, fasting, and KD induce lipolysis in adipose tissue to increase circulating FFAs for ketone production, and this is normally suppressed by insulin secretion from pancreatic β cells [104]. Finally, host–microbe crosstalk may also crucially contribute to diet-induced ketogenesis and stemness because the gut microbiome helps to generate short-chain fatty acids (SCFAs) by fermenting dietary fibers to suppress insulin-mediated fat accumulation [105] and supply the substrates for ketone body production [106]. However, how the gut microbiota mechanistically meditates diet-induced ISC fates through ketogenesis remains unclear. Collectively, these considerations underscore the complexity of diet-induced physiology and encourage future studies to perform systematic surveys using standardized dietary models, connect different dimensions of dietary responses, and ultimately provide more integrative dietary suggestions.

Outstanding Questions

In addition to calories, what are the essential dietary formulations that need to be defined and standardized for future dietary research?

How do acute and chronic dietary responses differentially influence ISCs through diet-derived nutrients and diet-induced endocrinology?

To what extent does organismal endocrinology influence cell metabolism and regulate ISC adaptation to diet?

How does gut microbiota mechanistically meditate diet-induced ISC fates?

Do diets differentially regulate normal and malignant ISCs, and how do they do so?

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