

Dietary *Aloe vera* gel and Microbiota Interactions: Influence of Butyrate and Insulin Sensitivity

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ABSTRACT

Symbiotic effect of butyrate-producing endophytic microbiota and *Aloe vera* gel containing non-digestible carbohydrates was discussed on slowing ageing design: butyrate efficacy for insulin sensitivity, sirtuin activation through histone deacetylase inhibitors *in vitro* study. Possible putative efficacy of butyrate fermented by endophytic microbiota for insulin sensitivity on glucose homeostasis is discussed.

Key words: Symbiosis; Dietary *Aloe vera* gel; Butyrate fermentation; Insulin sensitivity

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INTRODUCTION

Diet is one of the most influential lifestyle factors contributing to the rise of inflammatory disease and autoimmune diseases. Metabolism and immunity are inextricably linked to each other and to the organismal function, allowing adaptation to diverse changes in both internal and external environments. Possible efficacy of long-term ingestion of *Aloe vera* gel metabolites on insulin sensitivity has been proposed by Yagi A^[1], and the brain-liver-intestine circuit was considered for coordination between insulin sensitivity and glucose production. On exploration of health maintenance with *Aloe vera* gel long-term ingestion, Yagi A group^[2] proposed that the dietary natural source of butyrate produced by fermentation of acemannan in *Aloe vera* gel, is a highly appealing approach to present a simple and relatively low risk method to potentially improve outcomes in aged people with brain troubles. Krautkramer KA group^[3] demonstrated that gut microbiota alter host histone acetylation and methylation in the multiple host tissues; the proximal colon, the liver and fat tissue. Changes in the diet affect epigenetics via the microbiota and prevent many of the microbiota-dependent chromatin changes that occur in polysaccharide-rich diet. In preclinical study, Gao Z group^[4] showed that dietary supplementation of butyrate can prevent and treat diet-induced obesity and insulin resistance in mouse models. In our earlier report^[5], *Aloe vera* gel fermentation with endophytic microbiota stimulates microbiota-driven short chain fatty acids (SCFAs); butyrate production, and daily intake of the butyric acid fermentation extract may provide the possible potential preventive and therapeutic roles in human health. Here, we discuss a possible contribution of butyrate, as a histone deacetylase inhibitors and insulin sensitivity to age-related muscle atrophy, slowing ageing design and symbiotic

effect of *Aloe vera* endophytic microbiota with dietary *Aloe vera* ingestion.

IDENTIFICATION OF BUTYRATE-PRODUCING ENDOPHYTIC MICROBIOTA AND FINDING OF ANTI-INFLAMMATORY ACTIVITIES OF BUTYRATE IN ALOE VERA GEL FERMENTED MEDIA

Following microbiota: *Bacillus cereus*, *B. licheniformis*, *Lactobacillus paralimentarius* and *Clavispora lusitaniae* were identified by Al-Madboly L group^[6] from the fermentation of endophytic bacteria in *Aloe vera* gel by using MALDI TOF/MS analysis. The extracted fraction from fermented medium of *Aloe vera* gel showed abilities to regulate the inflammatory responses in the inflammation cell models via the reduction in amount of induced SOD and COX1 & 2 enzymes. The finding of anti-inflammatory activities of butyrate in the fermented medium may help explain the known beneficial effects of butyrate in intestinal colon and on colitis.

POSSIBLE EFFICACY OF SODIUM BUTYRATE FOR INSULIN SENSITIVITY

Lin HV group^[7] examined the effect of short chain fatty acid administration in mice, and showed that butyrate, propionate, and acetate all protected against diet-induced obesity and insulin resistance. Butyrate and propionate, but not acetate, induce gut hormones and reduce food intake. Stimulation of gut hormones and food intake inhibition by butyrate and propionate may represent a novel mechanism by which gut microbiota regulates host metabolism. Butyrate is a natural nutrient existed in food and can be produced physically through the intestinal fermentation of fiber. Pregnancy and obesity model would be helpful for understanding how β -cell adapts to insulin resistance and how butyrate alleviates the metabolic impairment and protects pancreatic β -cell function in pregnant mice with obesity. Li HP group^[8] found that mice fed with high sodium butyrate food showed obviously changes including the decreased values of weight gain, glucose, insulin, triglyceride and total cholesterol level of blood compared with high fat diet group. Mice fed with high sodium butyrate food group displayed β -cell hyperplasia with a greater cell size and β -cell area in pancreas. Butyrate shows an obvious function of anti-obesity, and can alleviate the metabolic stress, maintain the β -cell function and protect them from inflammatory response in pregnant obese mouse without obvious fetus toxicity. Gao Z group^[4] investigated that supplementation of butyrate in the obese mice led to an increase in insulin sensitivity and a reduction in adiposity, and the mechanism of butyrate action is related to promotion of energy expenditure and induction of mitochondria function. In an earlier review, it was reported that the metabolites of aloe polymannose moiety, such as manno-oligosaccharide and short chain fatty acids, synergistically modulate insulin sensitivity on tissues with combination of phenolics in *Aloe vera* gel. Henagan TM group^[9] determined a possible mechanism of sodium butyrate (NaB) action through induction of beneficial skeletal muscle mitochondrial adaptations and applied micrococcal nuclease digestion with sequencing to assess whole genome differences in nucleosome occupancy or positioning and to identify associated epigenetic targets of NaB. Treatment with NaB may be an effective pharmacological approach for type 2 diabetes and obesity by reducing-1 nucleosome repositioning within

nuclear-encoded mitochondrial genes, causing skeletal muscle mitochondrial adaptations that result in more complete β -oxidation and a lean, insulin sensitive phenotype. Zhou D group^[10] investigated whether gut microbiota metabolite, NaB is an effective substance for attenuating non-alcoholic fatty liver disease (NAFLD) and the internal mechanisms. NaB corrected the high-fat diet-induced gut microbiota imbalance in mice, while it considerably elevated the abundances of the beneficial bacteria *Christensenellaceae*, *Blautia* and *Lactobacillus*. NaB may restore the dysbiosis of gut microbiota to attenuate steatohepatitis, which is suggested to be a potential gut microbiota modulator and therapeutic substance for NAFLD. Butyrate produced by fermentation in large intestine by gut microbiota has been demonstrated to be protective against insulin resistance and fatty liver. Mollica MP group^[11] identified mitochondria as the main target of the beneficial effect of butyrate and its synthetic derivative, N-(1-carbamoyl-2-phenyl-ethyl) butyramide, FBA, in reverting insulin resistance and fat accumulation in diet-induced obese mice. The authors concluded that butyrate and FBA modulating mitochondrial function, efficiency, and dynamics, can be considered a new therapeutic strategy to counteract obesity and insulin resistance. Sarcopenia, the loss of skeletal muscle mass and function during ageing, is a major contributor to disability and frailty in the elderly. Walsh ME group^[12] investigated the potential for histone deacetylase (HDAC) inhibitor butyrate to modulate age-related muscle loss. They reported a beneficial effect of butyrate on muscle mass during ageing and suggested that HDACs contribute to age-related muscle atrophy and may be effective targets for intervention in sarcopenia and age-related metabolic disease. Canfora EE group^[13] discussed the effects of SCFAs on energy homeostasis and metabolism, as well as how SCFAs can beneficially modulate adipose tissue, skeletal muscle and liver tissue function, and resulted that SCFAs contribute to improved glucose homeostasis and insulin sensitivity. Kimura I group^[14] showed the short chain fatty acid receptor; G-protein coupled receptor: GPR43, links the metabolic activity of the gut microbiota with host body energy homeostasis. The authors demonstrated that GPR43-deficient mice are obese on a normal diet, whereas mice overexpressing GPR43 specifically in adipose tissue remain lean even when fed a high-fat diet. Furthermore, the authors showed SCFA-mediated activation of GPR43 suppresses insulin signaling in adipocytes, which inhibits fat accumulation in adipose tissue and promotes the metabolism of unincorporated lipids and glucose in other tissues. These findings establish GPR43 as a sensor for excessive dietary energy, thereby controlling body energy utilization while maintaining metabolic homeostasis. Khan S., Jena G^[15] evaluated the comparative effects of NaB and metformin (Met) on the glucose homeostasis, insulin-resistance, fat accumulation and dyslipidemia in type-2 diabetic rat. The authors demonstrated that NaB and Met reduced insulin-resistance, dyslipidemia, fat accumulation and gluconeogenesis thereby improved the glucose homeostasis in rat. NaB might be a promising molecule for the prevention and treatment of type-2 diabetes and dyslipidemia.

HDAC INHIBITOR: BUTYRATE IN CLINICAL APPLICATIONS

Histone deacetylase (HDACs) can deacetylate the histone, various transcription factors and regulatory proteins, which directly or indirectly affect glucose metabolism. There is a link between diabetes mellitus (DM) and HDACs, because HDACs inhibitors promote β -cell differentiation, proliferation, function and improve

insulin resistance. Butyrate and butyrate-producing microbes are decreased in DM. Khan S., Jena G^[16] provide a programmatic interpretation of chromatin-dependent and independent complex signaling/mechanisms of butyrate for the treatment of type 1 and 2 DM, with an emphasis on the promising strategies for its drugability and therapeutic implication. HDAC inhibitors interfere with HDAC activity and regulate biological events, such as cell cycle, differentiation and apoptosis in cancer cells.

The mammalian intestines contain an enormous number of microorganisms within the lumen. Given the constant exposure to these microbes, the intestinal immune system has the difficult task of maintaining tolerance to commensal bacteria while remaining responsive to potential pathogens. The mechanisms by which this balance is achieved are relatively unknown. Chang PV group^[17] identified a bacterial metabolite, butyrate that exerts immunomodulatory effects on intestinal macrophages and renders them hypo-responsive to commensal that reside in the colon. The authors elucidated a possible mechanism that contributes to immune homeostasis in the intestines. To date, the FDA has approved three HDAC inhibitors for cutaneous/peripheral T-cell lymphoma and many more HDAC inhibitors are in different stages of clinical development for the treatment of hematological malignancies as well as solid tumors. Mottamal M group^[18] summarized four major structural classes of HDAC inhibitors that are in clinical trials and different computer modeling tools available for their structural modifications as a guide to discover additional HDAC inhibitors with greater therapeutic utility.

There are results suggesting that butyrate, at least partly, explains the association between the intestinal ecosystem and host metabolic health. Real-time PCR analysis and in-depth microbiome sequencing have revealed lower abundance of butyrate-producing bacteria in patients with T2D. Interventions with butyrate *per se* or modulation of the intestinal microbiota to increased growth of butyrate producers have been associated with beneficial effects on insulin sensitivity both in mice and in human. Brahe LK group^[19] concluded that butyrate provides a link between diet, intestinal microbiota and metabolic health. Richards JL group^[20] summarized how diet, microbiota and gut microbial metabolites, (particularly SCFAs) can modulate the progression of inflammatory diseases and autoimmunity, and reveal the molecular mechanisms (metabolite-sensing G protein-coupled receptor and inhibition of histone deacetylases). Sathishkumar C group^[21] investigated the extent of HDAC machinery and inflammatory signals in peripheral blood mononuclear cells (PBMCs) from patients with type 2 diabetes mellitus (T2DM) compared to control subjects. Transcriptional levels of DBC1 (deleted in breast cancer 1, which is a negative regulator of HDAC3) were seen significantly reduced in PBMCs from T2DM. HDAC3 activity/HDAC3 mRNA levels positively correlated to pro-inflammation, poor glycemic control, and insulin resistance. Xiong H group^[22] proposed a new mechanism that butyrate manipulates endogenous host defense peptide (HDP) which contributes to the elimination of *Escherichia coli* O157:H7, and thus affects the alleviation of inflammation. The findings indicate that butyrate enhances disease resistance, promotes the clearance of *E.coli* O157:H7, and alleviates the clinical symptoms of *E.coli* O157:H7-induced hemolytic uremic syndrome and inflammation, partially, by affecting host defense peptide expression via HDAC inhibition.

As interest in the gut microbiome has grown in recent years, attention has turned to the impact of our diet on our brain. Bourassa MW group^[23] reviewed butyrate produced by bacterial fermentation of fiber in the colon, can improve brain health. Butyrate has been

extensively studied as a HDAC inhibitor but also functions as a ligand for a subset of G protein-coupled receptors and as an energy metabolite. The authors integrated evidence from the disparate fields of gastroenterology and neuroscience to hypothesize that the metabolism of high fiber diet in the gut can alter gene expression in the brain to prevent neuro-degeneration and promote regeneration. Increasing evidence suggests that alterations in epigenetic mechanisms regulating chromatin state play a role in the pathogenesis of medulloblastoma (MB), the most common malignant brain tumor of children. HDAC inhibitor, sodium butyrate (NaB), which increases chromatin relaxation, has been shown to display anticancer activity. NaB markedly increases cell death and reduces colony formation in human MB cell lines, and displays pronounced inhibitory effects on the survival of human MB cells. Nor C group^[24] shows that HDAC inhibition might promote the neuronal differentiation of MB cells and provides the first evidence that an HDAC inhibitor, NaB, might suppress the expansion or survival of MB cancer stem cells. Stilling RM group^[25] provided a critical review on butyrate and its effects on multiple aspects of host physiology with a focus on brain function and behavior. The authors showed fundamental differences in natural butyrate at physiological concentrations and its use as a neuro-pharmacological agent at rather high, supra-physiological doses in brain research. The authors hypothesize that butyrate and other volatile SCFAs produced by microbes may be involved in regulating the impact of the microbiome on behavior including social communication. Montiel F group^[26] examined the regulation of the insulin receptor by butyrate using ¹²⁵I-insulin in C6 cells (a rat glioma cell line). The authors indicated that C6 cells bind insulin specifically and that butyrate and SCFAs down-regulate surface insulin-receptor number. Incubation with butyrate results in a time- and dose-dependent decrease of insulin binding to C6 cells. Garcia-Caceres C group^[27] reported astrocytic insulin signaling co-regulates hypothalamic glucose sensing and systemic glucose metabolism. Postnatal ablation of insulin receptors (IRs) in glial fibrillary acidic protein (GFAP)-expressing cells affects hypothalamic astrocyte morphology, mitochondrial function, and circuit connectivity. Accordingly, astrocytic IR ablation reduces glucose-induced activation of hypothalamic pro-opiomelanocortin neurons and impairs physiological responses to changes in glucose availability. A normal response to altering directly CNS glucose levels in mice lacking astrocytic IRs indicates a role in glucose transport across the blood-brain-barrier (BBB). This was combined *in vivo* in GFAP-IR KO mice by using positron emission tomography and glucose monitoring in cerebral spinal fluid. The authors concluded that insulin signaling in hypothalamic astrocytes co-controls CNS glucose sensing and systemic glucose metabolism via regulation of glucose uptake across the BBB.

Dinan TG, Cryan JF^[28] suggest that the gut microbiota has been implicated in a variety of conditions including depression, autism, schizophrenia and Parkinson's disease. There is still considerable debate as to whether or not the gut microbiota changes are core to the pathophysiology of such conditions or merely epi-phenomenal. It is plausible that such neuropsychiatric disorders might be treated in the future by targeting the microbiota either by microbiota transplantation, antibiotics or psychobiotics.

A large number of clinical trials have been completed and are going on in both hematological and solid malignancies using a wide variety of HDAC inhibitors as well as in combination with other agents. Understanding the mechanisms of HDAC inhibitor-induced cell death is essential and this will aid the search for predictive biomarkers. Multiple beneficial effects of butyrate at intestinal and extra-

intestinal level have been demonstrated and the mechanisms of action of butyrate are different and many of these involve an epigenetic regulation of gene expression through the inhibition of HDAC. These findings provide the basis for future clinical studies aiming to elucidate the potential preventive and therapeutic roles of butyrate in human medicine.

Sir2 deacetylase of yeast; *Clavispora lusitaniae* (*Saccharmycetales*: genbank synonyms of *Candida lusitaniae*; teleomorph *Clavispora lusitaniae*)

The types and concentration of the volatile compounds are significantly influenced by the species of microorganisms, and the corresponding representative fungi, in the wheat Qu samples were investigated by Yu L group^[29]. As one of the yeast, *Clavispora lusitaniae* produced high concentration of the total CFUs in test samples, and the GC-MS analysis of the volatile compounds showed alcohols, esters and acids as main flavor substances produced by the yeast. Aroma compounds in Chinese rice wines were studied by Fan W. and Xu Y^[30] using GC-olfactometry (GC-O) and GC-MS spectrometry. Fifty seven-aroma compounds were identified by GC-O followed by GC-MS, among which butyric acid, other alcohols and esters were identified with the highest aroma intensities. The results indicated that the richer the flavor substances were in the rice wines, the more abundant the microbe species in the corresponding wheat Qu samples.

Centromeres are the site where chromosomes attach to microtubules during mitosis, and they are necessary for chromosome segregation. Kapoor S group^[31] discovered an unusual type of centromere in the yeast, *Clavispora lusitaniae*, which is an occasional human pathogen. These centromeres are similar to many eukaryotic centromeres in that they are not specified by a particular sequence. *Clavispora lusitaniae* centromeres are not flanked by a compact chromatin structure, known as pericentromeric heterochromatin. This finding reveals that although pericentromeric heterochromatin is generally important for promoting proper centromere function, it is not universally necessary. This unusual centromere structure could contribute to stress-induced errors in chromosome segregation that are observed in *Candida* species.

Sirtuins were initially found to slow ageing in lower organisms and more recently shown to mediate many effects of calorie restriction on metabolism and longevity in mammalian, and are a family of nicotinamide adenine dinucleotide (NAD⁺)-dependent protein deacetylases that are evolutionary conserved from yeast to human. The mammalian sirtuin family has attracted tremendous attention over the past few years as stress adaptors and post-translational modifier. They have involved in diverse cellular processes including DNA repair, energy metabolism, and tumorigenesis. Sirt1 (silent information regulator-1) induces histone deacetylation and methylation, promoter CpG island methylation, transcriptional repression, and deacetylation of tumor suppressor proteins. Deacetylases of the Sirt 2 (silent information regulator-2) or sirtuin family are an important family of protein through to regulate life cycle progression and life span in response to nutrient availability. These proteins have diversified over the course of evolution such that humans possess seven family members. Froyd CA. group^[32] examined the sirtuin called Sir2 ortholog ClHst 1 in the yeast *Clavispora lusitaniae*, and demonstrated that it has lost the capacity to act at telomeres, which are the ends of chromosomes. The authors have identified an opportunity to discover how organisms adapt to the loss of an evolutionarily conserved function. Thus, *Clavispora lusitaniae* presents an opportunity to discover how subtelomeric chromatin can be re-configured.

SLOWING AGEING DESIGN: SIRTUIN ACTIVATION

Yeast Sir2 is a heterochromatin component that silencing transcription at silent mating loci, telomeres and the ribosomal DNA, and that also suppresses recombination in the rDNA and extends replicative life span. Mutational studies by Imai S. group^[33] indicated lysine 16 in the amino-terminal tail of histone H4 and lysine 9, 14 and 18 in H13 are critically important in silencing, whereas lysine 5, 8, and 12 of H4 have more redundant functions. Lysine 9 and 14 of histone H3 and lysine 5, 8 and 16 of H4 are acetylated in active chromatin and hypo-acetylated in silenced chromatin, and overexpression of Sir2 promotes global deacetylation of histones, indicating that Sir2 may be a histone deacetylase. The authors showed that yeast and mouse Sir2 proteins are nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylases, which deacetylate lysine 9 and 14 of H3 and specifically lysine 16 of H4. The analysis of two Sir2 mutation supports the idea that this deacetylase activity accounts for silencing, recombination suppression and extension of life span *in vivo*. Furthermore, Imai S^[34] provided a proof of concept for the idea that nicotinamide mononucleotide, the nicotinamide phosphoribosyl-transferase reaction product, can be used as a nutraceutical to activate Sirt1 activity.

Mice engineered to express additional copies of SIRT1 or SIRT6, or treated with sirtuin-activating compounds (STACs) such as resveratrol and SRT1 or SIRT2104 or with NAD⁺ precursors, have improved organ function, physical endurance, disease resistance and longevity. Bonkowski MS., Sinclair DA^[35] discussed that trials in non-human primates and in humans have indicated that STACs may be safe and effective in treating inflammatory and metabolic disorders, among others. These advances have demonstrated that it is possible to rationally design that can alleviate multiple diseases and possibly extend lifespan in human. The multifunctional SIRTs regulate cellular and whole-body metabolism processes, such as cell survival, glucose/lipid metabolism, mitochondrial biogenesis and DNA stabilization and repair. Resveratrol, a well-known SIRT1 activator, helps prevent metabolic disorder by increasing glucose utility and insulin sensitivity, and improves survival of mice fed a high-calorie diet. Yagi A, Yu BP^[36] reported that the health-promoting potential of a balanced gut microbiota status modulated by calorie restriction posits a possible close link between gut microbiota and healthy ageing. Intestinal microbiota producing butyrate might be developed for the therapeutic purpose to increase insulin sensitivity in humans with calorie restriction. Park S group^[37] described that the numerous studies discussed support a close involvement of Sirt2/SIRT1 in calorie restriction (CR)-mediated lifespan extension and suggested the possibility that SIRT1 may not be the only factor, but rather one of several components that regulate CR-mediated lifespan extension, as neither overexpression of SIRT1 nor resveratrol extended lifespan in mammals. Guarente L^[38] speculated on how an increase in mitochondrial activity might provide benefit and discuss how diet, mitochondria, and sirtuins might interact in a pathway to slow ageing and associated diseases. Furthermore, Guarente L^[39] discussed emerging data showing protection by sirtuins against most of the common diseases of ageing. The author showed possibility that sirtuins will be novel targets to combat these diseases pharmacologically. Leukocyte telomere length is widely considered a biomarker of human age and in many studies indicative of health or disease. Kim S group^[40] indicated quantitative estimates of telomere length from blood leukocytes in a population sample, and confirmed results showing telomere length significantly decreases with age.

The authors suggested a potential role of SIRT1 in linking telomere length and human longevity. Kitada M group^[41] demonstrated the study on calorie restriction (CR) in four overweight male participants with 25% CR of their baseline energy requirements for 7 weeks. The authors assessed the effects of the serum collected from the participants on AMP-activated protein kinase (AMPK) and SIRT1 activation and mitochondrial biogenesis in cultured human skeletal cells. The skeletal muscle cells that were cultured in serum collected after CR showed an increase in AMPK and SIRT1 activity as well as mitochondrial biogenesis. The authors discussed that CR is beneficial for obesity-related metabolic alterations and induces cellular adaptations against ageing, possibly through AMPK and SIRT1 activation via circulating factors.

Lee HJ., Yang SJ^[42] identified the pattern of serum SIRT1 activity according to age and sex, and investigated how serum SIRT1 activity is correlated with other metabolic parameters in Korean adults. Positive correlation was observed between SIRT1 activity and basal metabolic rate in women ($r = -0.183$, $p = 0.027$), but not in men, suggesting that serum SIRT1 activity may reflect energy expenditure well in human and the possibility that serum SIRT1 activities may be utilized as a biomarker of ageing.

Butyrate is a confirmed inhibitor of class I and II HDACs and is among the family of small-molecule HDACs inhibitors that are known to inhibit cancers but the underlying anticancer effects of butyrate remain elusive. Xu S group^[43] reported that butyrate promotes cancer cell apoptosis by acting as a SIRT3 inhibitor. Butyrate inhibits SIRT3 both in cultured cells and *in vitro*. Butyrate-induced pyruvate dehydrogenase E1- α (PDHA1) hyper-acetylation relieves the inhibitory phosphorylation of PDHA1 at serine 293, thereby activating an influx of glycolytic intermediates into the tricarboxylic acid cycle and reversing the Warburg effect. [PDHA1: the first component enzyme of the pyruvate dehydrogenase (PDH) complex (PDC) that transforms pyruvate via pyruvate decarboxylation, into acetyl CoA that is subsequently used by both the citric acid cycle and oxidative phosphorylation to generate ATP.] Butyrate-induced hyper-acetylation inactivates complex I of the electron transfer chain and prevents the utilization of TCA cycle intermediates. These metabolic stresses promote apoptosis in hyper-glycolytic cancer cells, such as HCT116p53^{-/-} cells, SIRT3 deacetylates both PDHA1 and complex I. Genetic ablation of SIRT3 in mouse hepatocytes abrogated the ability of butyrate to induce apoptosis. The results identify a butyrate-mediated anti-tumor mechanism and indicate that the combined activation of PDC and inhibition of complex I is a novel tumor treatment strategy. Jung HY group^[44] investigated the effects of the sirtuin-2 (SIRT2) inhibitor AK-7 (a neuroprotective in Huntington disease) on novel object memory, cell proliferation, and neuroblast differentiation in the dentate gyrus. The authors observed the relationships with sodium butyrate, a histone deacetylase inhibitor, on the hippocampal functions. The results suggested that the reduction of SIRT2 may be closely related to age-related phenotypes including novel object memory as well as cell proliferation and neuroblast differentiation in the dentate gyrus and sodium butyrate reverses SIRT2-related age phenotypes. Histone acetylation and deacetylation are key factors modulating hippocampal functions, such as memory formation in the dentate gyrus. Yoo DY group^[45] investigated the role of SIRT2 in cell proliferation and neuroblast differentiation in the mouse dentate gyrus, and suggested that histone acetylation and deacetylation are key factors modulating hippocampal functions such as memory formation, cell proliferation, and neuroblast differentiation in the dentate gyrus.

Gluconeogenesis is a ubiquitous and endogenous glucose synthetic process present in plants, animal, fungi and bacteria. Intestinal gluconeogenesis (IGN) has beneficial effects on glucose and energy homeostasis. De Vadder F group^[46] showed that propionate and butyrate, which are generated by fermentation of soluble fiber by the gut microbiota, activate IGN via complementary mechanisms. Butyrate activates IGN gene expression through a cAMP-dependent mechanism, while propionate, itself a substrate of IGN, activates IGN gene expression via a gut-brain neural circuit involving the fatty acid receptor FFAR3. The metabolic benefits on body weight and glucose control induced by SCFAs or dietary fiber in normal mice are absent in mice deficient for IGN, despite similar modifications in gut microbiota composition. Thus, the regulation of IGN is necessary for the metabolic benefits associated with SCFAs and soluble fiber.

SYMBIOTIC EFFECT OF ALOE VERA GEL WITH ALOE ENDOPHYTIC MICROBIOTA

In an earlier report possible putative efficacy of *Aloe vera* gel metabolites in long-term ingestion to insulin sensitivity was discussed, and an assumption based on a brain-liver-intestine circuit to coordinate with insulin sensitivity of endogenous glucose production was proposed^[1].

Symbiosis by prebiotics and probiotics plays an important role in maintaining normal physiological status of biological organisms with natural resistance, and intestinal microbiota perturbation may potentially lead to disease status. Gut microbiota have critical interactions with the host immune system and metabolism.

Liu Y group^[47] aimed to assess the impact of various fermentable carbohydrates, such as trans-galacto-oligosaccharides and inulin, and microbiota-organisms on gut microbiota and selected immune markers, using anaerobic batch culture fermenters to the elderly volunteers. The impact of fermentation supernatants on immune markers relevant to the elderly were assessed *in vitro*. Supernatants from prebiotic and probiotic fermentations showed an anti-inflammatory effect by inhibiting production of pro-inflammatory cytokines and enhancing production of anti-inflammatory cytokines which was possibly related to short chain fatty acids, including butyric acid concentrations. They concluded that the symbiotic could lead to potentially beneficial effects to host health by targeting specific bacterial groups, increasing saccharolytic fermentation and decreasing inflammation associated with ageing. In our early report^[48], symbiotic effect of *Aloe vera* juice on growth of *Lactobacillus fermentum*, acetic, propionic and lactic acid were identified as short chain fatty acid (SCFA), and only butyric acid was found in the fermented broth of *Aloe vera* leaf gel with endophytic bacteria. Yadav H group^[49] demonstrated that the administration of a probiotic, VSL#3, containing few strains of *Lactobacillus* and *Bifidobacterium*, prevented and treated obesity and diabetes in several mouse models. VSL#3 suppressed body weight gain and insulin resistance via modulation of the gut flora composition. Moreover, VSL#3 promoted the release of the hormone glucagon-like peptide (GLP-1), resulting in reduced food intake and improved glucose tolerance and prevented obesity and diabetes in mice via induction of butyrate and GLP-1. The VSL#3-induced changes were associated with an increase in the levels of SCFA, butyrate. The authors suggested that probiotics can modulate the gut-microbiota-SCFA-hormone axis and are of potential therapeutic utility to counter obesity and diabetes. The effects of the probiotic, VSL#3, on body and fat mass, insulin sensitivity, and skeletal muscle substrate oxidation following 4 weeks of a high-fat diet in twenty non-obese male (18-30 years-participants) were

determined by Osterberg KL group^[50]. Body mass and fat mass increased less following the high-fat diet in the VSL#3 compared with placebo. However, there were no significant changes in insulin sensitivity or *in vivo* skeletal muscle pyruvate and fat oxidation with the high-fat diet or VSL#3. VSL#3 supplementation appears to have provided some protection from body mass gain and fat accumulation in healthy young men consuming a high-fat and high-energy diet. Pingitore A group^[51] evaluated the effects of increasing colonic delivery of the SCFA propionate on β -cell function in humans and the direct effects of propionate on isolated human islets *in vitro*. The results indicated that propionate has beneficial effects on β -cell function *in vivo*, and *in vitro* analyses demonstrated that it has direct effects to potentiate glucose-stimulated insulin release and maintain β -cell mass through inhibition of apoptosis. These observations support ingestion of propionate dietary fibers to maintain healthy glucose homeostasis.

Recent studies involving microbiota-based interventions in human underscores that an altered microbiota may directly modulate host metabolism in humans. However, it will be essential to determine whether an altered gut microbiota precedes development of insulin resistance and diabetes and to identify the underlying molecular mechanisms. Increased mechanistic insights of how the microbiota modulates metabolic disease in humans may pave the way for identification of innovative microbiota-based diagnostics and/or therapeutics (probiotic bacterial strains).

SUMMARY

The evaluation of the prebiotic potential of *Aloe vera* gel rich in a polysaccharide, acemannan, was carried out by *in vitro* fermentation of the endophytic microbiota: *Lactobacillus paralimentarius*, *Bacillus cereus*, *Bacillus licheniformis* and *Clavispora lusitanae*. Butyric acid was identified as one of short chain fatty acid (SCFA) in the fermented extract and the anti-inflammatory activity of the extract was assessed by regulation of the inflammatory responses in the inflammation cell models via the reduction in amount of induced SOD and COX 1 & 2 enzymes.

On symbiotic effect of *Aloe vera* juice on growth to *Lactobacillus fermentum* and *L. helveticus* isolates, and endophytic microbiota in *Aloe vera* gel, acetic, propionic and lactic acid in *L. fermentum* fermentation and butyric acid in the endophytic microbiota fermentation were respectively identified as SCFAs. The significant importance of probiotics compared to prebiotics was strongly indicated. Butyrate is a functionally versatile molecule that is produced in the mammalian gut by fermentation of dietary fiber and is enriched in butter and other dairy products. Butyrate along with other fermentation-derived SCFAs shows promising effects in various diseases as well as neurological disorders. The dietary sources of butyrate through a high fiber diet or a diet rich in natural sources of butyrate are a highly appealing approach, as it presents a simple and relatively low risk method to potentially improve outcomes in aged patients with brain disorders.

An innovative concept of symbiotic: a combination of *Aloe vera* gel and microbiota is a perspective on modulation of insulin sensitivity and improvement of gastro-intestine health by butyrate fermentation.

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