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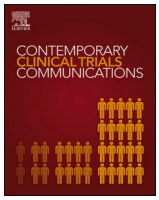

Can butyrate prevent colon cancer? The AusFAP study: A randomised, crossover clinical trial

Julie Clarke [a,*], Alex Boussioutas [b], Brooke Flanders [b,1], Trevor Lockett [c,2], Karen Harrap [d], Ian Saunders [e], Patrick Lynch [f], Mark Appleyard [g], Allan Spigelman [h], Don Cameron [i], Finlay Macrae [b]

[a] CSIRO Health and Biosecurity Adelaide, SA, 5000, Australia
[b] Department of Medicine, Royal Melbourne Hospital, University of Melbourne, Victoria, 3050, Australia
[c] CSIRO Health and Biosecurity, North Ryde, NSW, 1670, Australia
[d] CSIRO Health and Biosecurity, Herston, Brisbane, Queensland, 4006, Australia
[e] CSIRO Computational Informatics, Glen, Osmond, SA, 5064, Australia
[f] Division of Internal Medicine, MD Anderson Cancer Center, Houston, TX, 77030, USA
[g] Royal Brisbane & Women's Hospital, Herston, Queensland, 4029, Australia
[h] University of New South Wales and St Vincent's Hospital, Sydney, New South Wales, 2010, Australia
[i] Royal Children's Hospital, Melbourne, Victoria, 3052, Australia

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ABSTRACT

Increased colonic butyrate from microbial fermentation of fibre may protect from colorectal cancer (CRC). Dietary butyrylated high amylose maize starch (HAMSB) delivers butyrate to the large bowel. The objective of this clinical trial (AusFAP) is to evaluate potential chemoprotective effects of HAMSB on polyposis in individuals with a genetic form of colon cancer, Familial Adenomatous Polyposis (FAP).

The study is a multi-site, double blind, randomised, placebo-controlled crossover trial undertaken at major hospitals in Australia. After a baseline endoscopy participants consume either 40g/day of HAMSB or placebo (low amylose maize) starch for 26 weeks. After another endoscopic examination participants consume the alternate starch for 26 weeks. A third endoscopy at 52 weeks is followed by 26 weeks' washout and a final endoscopy at 78 weeks. Primary outcome measure is the global large bowel polyp number. Secondary measures include global polyp size counts, and number and size of polyps at two tattoo sites: one cleared of polyps at baseline, and another safely chosen with polyps left in situ during the study. Other secondary outcome measures include the effects of intervention on cellular proliferation in colonic biopsies, faecal measures including short chain fatty acid concentrations, and participants' dietary intakes. Generalized linear mixed models analysis will be used to estimate differences in primary outcomes between intervention and placebo periods.

This study represents the first clinical evaluation of the effects of increased colonic butyrate on polyp burden in FAP which, if effective, may translate to lower risk of sporadic CRC in the community.

Australian New Zealand Clinical Trials Registry Number: 12612000804886.

1. Introduction

The incidence of colorectal cancer is increasing in most countries worldwide [1] and yet a large proportion of these cancers are preventable by modifiable lifestyle factors [2]. There is evidence that increasing consumption of dietary fibre and wholegrains decrease the risk of developing colon cancer [3] possibly by increasing production of butyrate from colonic fermentation of indigestible polysaccharides. Butyrate is a potent histone deacetylase inhibitor that modulates

* Corresponding author. CSIRO, PO Box 10041, Adelaide, BC 5000, SA, Australia.
E-mail addresses: julie.clarke@csiro.au (J. Clarke), alexb@unimelb.edu.au (A. Boussioutas), brooke.flanders@mh.org.au (B. Flanders), trevor.lockett@rhythmbio.com (T. Lockett), karen.harrap@csiro.au (K. Harrap), ianxcsiro@gmail.com (I. Saunders), plynch@mdanderson.org (P. Lynch), Mark.Appleyard@health.qld.gov.au (M. Appleyard), Allan.Spigelman@svha.org.au (A. Spigelman), Don.Cameron@rch.org.au (D. Cameron), Finlay.Macrae@mh.org.au (F. Macrae).
[1] Present addresses: Barwon Health, Geelong Victoria 3220.
[2] Present addresses: Rhythm Biosciences, Melbourne Victoria 3000 Australia.

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Abbreviations

AusFAP	Australian FAP study
HAMSB	butyrylated high amylose maize starch
IC	intact colon
IPAA	ileal pouch anal-anastomosis
IRA	ileo-rectal anastomosis
LAMS	low amylose maize starch
EPA	eicosapentaenoic acid
RS	resistant starch
DS	degree of substitution
eCRF	electronic case report form
SAE	serious adverse events
SA Pathology	South Australia Pathology
HDAC	histone deacetylase inhibitor
GPCRs	G protein coupled receptors
SCFA	short chain fatty acids
CSIRO	Commonwealth Scientific and Industrial Research Organisation
RMH	Royal Melbourne Hospital
RBWH	Royal Brisbane Women's Hospital
DSMC	Data Safety Monitoring Committee
MedDRA	Medical Dictionary for Regulatory Activities

expression of genes that regulate proteins involved in cellular apoptosis, cell cycle regulation and DNA repair that may protect from colorectal oncogenesis [4].

Butyrylated high amylose maize starch (HAMSB) is an acylated starch which delivers significant quantities of butyrate to the colon of animals [5] and humans [6,7]. Butyrate delivered by HAMSB induces apoptosis in the epithelium at the base of colonic crypts [8], opposes colonocyte DNA strand breaks [9] and reduces tumour burden [10] in carcinogen-treated rats. In healthy humans, dietary HAMSB protects the rectal mucosa against increased expression of oncogenic microRNAs [11] and levels of mutagenic DNA adducts [12] associated with consuming a high red meat diet. However butyrate is a preferred energy source for colonocytes [13] and prolonged exposure to high levels of butyrate may result in resistance of cancer cells to the protective effects of butyrate [14]. *In vitro* studies with human colon cancer cells suggest this may be mediated through the downregulation of the AMP-activated protein kinase pathway [15].

Individuals with Familial Adenomatous Polyposis (FAP) have a rare, inherited germ-line *APC* mutation which results in the development of many colorectal adenomas and a high risk of developing colorectal cancer. Gene carriers have been used for testing the efficacy of potential chemo-preventative agents as FAP provides a well-recognised model of colorectal carcinogenesis [16] with mutations responsible for their condition also occurring in the majority of sporadic disease. Although many FAP patients undergo colonic resection with ileo-rectal anastomosis (IRA), or ileal pouch anal-anastomosis (IPAA) to reduce their risk of CRC, the microbiota within their residual bowel remains capable of releasing esterified butyrate from HAMSB [17].

The objective of this clinical trial ("AusFAP") is to determine if butyrylated starch has a chemoprotective effect on polyposis in FAP participants by reducing the incidence and/or growth of large bowel or pouch polyps. The study has a randomised, cross-over design and involves supplementation with HAMSB or placebo starch (low amylose maize starch, LAMS) for two 26-week interventions, followed by a 26-week washout period. Video recorded sigmoidoscopy/colonoscopy are undertaken at baseline and the end of each 6-month period enabling assessment of polyp burden and collection of biopsies for measurement of cellular kinetics and markers of colorectal cancer risk.

2. Materials and methods

2.1. Methods

2.1.1. AusFAP study design overview

AusFAP is a randomised, double-blind cross-over placebo controlled study of 18 months duration for each participant (Fig. 1). Endoscopic examinations are undertaken at baseline after which participants ingest either HAMSB or LAMS for 26 weeks (±2 weeks) and undergo a second endoscopy. They then consume the alternate starch for 26 weeks (±2 weeks) and have a third endoscopy at 52 weeks. This examination is followed by a 26-week (±2 week) washout during which no supplement is consumed, and a final fourth endoscopy at 78 weeks. Endoscopies are videoed and polyp and mucosal biopsy samples collected when available.

An intervention duration of 26 weeks or similar has been used previously in studies evaluating chemopreventive agents in FAP patients [18,19]. This time frame provides a balance between the time required for measurable polyp development or growth and participant compliance.

2.1.2. Participant recruitment strategy

Volunteers with medically diagnosed FAP and a history of polyp detection at any surveillance scopes are recruited if generally in good health, aged 12–75 and have either an intact colon (IC), or after colectomy, an ileo-rectal anastomosis (IRA), or ileal pouch anal-anastomosis (IPAA). The volunteers' genetic diagnosis (FAP/MUTYH mutation), if known, is recorded, along with incidence of desmoids, extra colonic manifestations, date of diagnosis and family history.

Inclusion/exclusion criteria are detailed in Table 1. Potentially eligible individuals are identified through state based FAP registries, as patients of Principal Investigators, from familial cancer centres, FAP self-help organisations, and from advertising. Participants are recruited at the Royal Melbourne (RMH), Cabrini and Royal Children's Hospitals in Melbourne; Royal Brisbane and Women's Hospital in Brisbane (RBWH); and St Vincent's Hospital in Sydney. The screening process involves consultations with both the Principal Investigator and clinical trial co-ordinators before informed consent is obtained from each participant prior to recruitment.

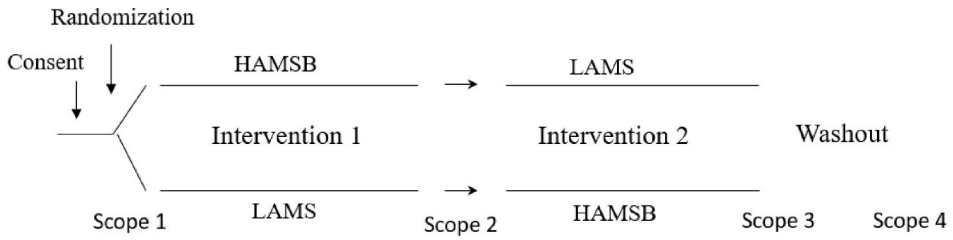
2.1.3. Interventions

Before commencing the AusFAP study a pilot trial was undertaken to determine if the large bowel microbiota of FAP participants who had undergone colectomy with IRA or IPAA are capable of releasing esterified butyrate. The study confirmed that a significant proportion of ingested esterified butyrate is released by the gut microflora in both groups of participants, and that 40g/day maximised faecal butyrate concentrations [17]. This dose of HAMSB is known to significantly increase free faecal butyrate in individuals with an intact gastrointestinal tract [7].

Participants in the AusFAP study consume either 40 g/day of HAMSB or LAMS (placebo) in two divided doses (20 g in the morning, 20 g in the evening) for 26 ± 2 weeks. LAMS is a commercially available readily digestible starch (Melogel, National Starch Food Innovation) that contains minimal quantities of resistant starch [6] (RS). National Starch Food Innovation (Ingredion Inc) manufactured the HAMSB using high resistant corn starch (Hylon VII) as the base starch. Starches were packaged into food grade, moisture resistant sachets containing 20 g ± 2g by Chippewa Packaging Inc (St Peter, MN, USA).

The degree of substitution (DS) of the HAMSB (the proportion of glucose hydroxyl groups in the base starch that were esterified with butyrate) was within specification (0.24–0.25) as measured using a titration technique [18]. The DS was also measured on HAMSB sampled from sachets prior to the commencement of the clinical trial, and annually during the clinical component of the study.

Independent quality control testing for microbial, yeast and heavy



Time (weeks)	0	4	8	12	16	26	30	34	38	42	52	78
Endoscopies[a]	x					x					x	x
24h recall[b]	x			x					x			
Gut symptom questionnaires	x			x		x			x		x	x
Height and weight measurement	x					x					x	x
24h faecal collections	x	x				x	x				x	
Blood sampling	x					x					x	x
Dispense test products	x		x		x	x		x		x		
Return unused sachets						x					x	

Fig. 1. Design and timeline of the AusFAP study
[a]The scopes were undertaken within ±2 weeks of from the scheduled date, [b]Week 0 dietary recall undertaken on day 3 or 4 post baseline endoscopy.

Table 1
Inclusion/exclusion criteria for the AusFAP clinical trial.

Category	Description
Inclusion	Age 12-75
	Medically diagnosed FAP with either an intact colon, or after colectomy with a residual rectum and ileorectal anastomosis or proctocolectomy with ileal pouch-anal anastomosis
	History of polyp detection at any surveillance sigmoidoscopies or colonoscopies
	Generally in good health
	Available for the duration of the study
Exclusion	Intolerant to high fibre products
	Reported lactating, pregnant or wish to become pregnant (including a male wishing to father a child) during the study. If a participant becomes pregnant during the trial they will be withdrawn
	Reported use of nonsteroidal anti-inflammatory drugs, probiotics or aspirin for two months prior to the trial. Note: If a patient requires aspirin for medical management (for example cardiovascular disease prophylaxis) they need to be on a stable dose for at least 2 months prior to randomisation and not alter the dose during the trial. If a short course of anti-inflammatories ≤10 days is required throughout the duration of the study, this exclusion will be waived.
	Use of other medication or supplement that, in the opinion of the gastroenterologist, may interfere with polyp development or bowel or microbiota function for 2 months prior to and during the clinical intervention. Use of anti-diarrhoeal medication(s) is allowed as required.
	Use of other experimental chemopreventative agents, including EPA, tumeric and curcumin for 6 months prior to and during the trial.
	Colonic or rectal surgery likely within 18 months.
	Has a stoma or ostomy.

metal contamination was undertaken by Gibraltar Laboratories Inc (NJ, USA) and SA Pathology (Adelaide, South Australia) on subsamples of the bulk packaged HAMSB. Additional testing was undertaken by SA Pathology on sachets of treatment and placebo starches before the commencement of the clinical trial, and annually from the date of manufacture during the study to ensure starch quality.

Each box of test starch is labelled with a 4-digit recruitment number randomly generated by the study eCRF, and a kit number (1–8), and dispensed to participants by either the hospital pharmacies, or the clinical co-ordinators.

During the baseline visit, participants are advised how to consume the test starches and are provided suggestions of what foods may be substituted for the supplements within their usual diets to avoid body weight changes. If significant weight changes unrelated to other clinical conditions are noted, further consultation with the research dietitian is provided to the participants.

2.1.4. Power calculations and randomisation

Sample size calculations are based on the change in global polyp number at endoscopy as this most closely relates to the primary end measure. Our estimates have been based on a cross-over design study [19] similar to the AusFAP trial. Using a conservative model independent of carryover or delayed effects and based on within-participant variability in polyp number at scopes after treatment 1 and 2, we estimated that, with a two-sided comparison and an alpha level of 0.05, the total number of participants required would be 64 (n = 32/group; 85% power, to detect a 23% difference in number of subjects with reduced polyps/total).

Randomisation is done centrally within the study eCRF which contains an algorithm that allocates the recruited participants to receive either intervention or placebo starch for consumption in the first 26 weeks of the study. The randomisation algorithm uses stratified allocation and considers participants' age (3 categories: 12–18, 19–45 and over 45 years), and their surgery type (3 types possible IC, IRA, IPAA). New participants are allocated to whichever treatment has the smaller number of participants previously allocated, thus ensuring approximately equal numbers in each treatment group.

2.1.5. Study protocol and measurements

The study design is detailed in Fig. 1. The two 26-week interventions are followed by a 26-week washout period and final endoscopy to determine if there are carryover effects from the dietary interventions. No six month washout between dietary treatments has been included as it was considered likely to result in a loss of participants to follow up. The gut microbiome adjusts rapidly to dietary alteration and a new gut environment will establish quickly after crossover, and will be maintained for the duration of the intervention in compliant participants.

The study participants, study coordinators and research team are blinded to the treatments throughout the study, and during data and sample analyses. An eCRF is used by the study co-ordinator to collect information and to enable assessment of videos by gastroenterologists at different clinical sites at the end of the study.

The primary outcome of the study is total polyp count as measured globally throughout the large bowel; secondary measures include size of the global polyps, and polyp number and size at two tattoo sites placed in the colon. To assess polyp burden, colonoscopies are undertaken on participants using high-definition endoscopy where possible. Ideally the same colonoscopist undertakes all the scopes on an individual. At the baseline endoscopy, two areas of mucosal surface approximately 36 mm in diameter (as measured by open biopsy forceps) and at least 50 mm apart are chosen for tattooing. The first site (tattoo 1) contains no polyps or is cleared of polyps at baseline scope to allow the rate of initiation of polyp growth to be assessed in subsequent scopes. The second site (tattoo 2) contains a small number of polyps at baseline to enable the growth or regression rate of polyps to be assessed in subsequent scopes. The centre point of each area is tattooed with Spot® Ex Endoscopic Tattoo, and the distance from the anal verge carefully measured. The size of polyps in tattoo 2 are carefully measured against open biopsy forceps and videoed, along with the large bowel mucosa, noting anatomical landmarks where evident. At subsequent colonoscopies, both tattoo sites and the large bowel are carefully videoed to enable later assessment. At each endoscopy, biopsies are taken of apparently normal mucosa and polyps collected for the study into RNA*later* and buffered formalin, or removed for clinical purposes if appropriate. With ethical approval, these samples may be used to determine the effects of butyrate on cellular proliferation and apoptosis in the colonic epithelium and adenoma and to quantify the level of dysregulated oncogenic miRNA in those tissues. Polyp removal or biopsy is videoed and then recorded in the eCRF.

Each video will be assessed by at least 2 independent gastroenterologists to determine the number and size of polyps in each segment of the large bowel, and at the two tattoo sites. The first assessor will review all the videos. The videos will be randomly assigned to the second assessors with all videos from particular participants assigned to the same second assessor. The baseline scope is identifiable (due to tattoo placement), but assessors are blinded to the sequence of scopes or treatments the participants received. The sizes of polyps are estimated against biopsy forceps and are binned into three size categories: $<$2.4 mm (less than the diameter of closed forceps), 2.4–9 mm (approximating or greater than the diameter of closed forceps but less than or equal to the diameter of open forceps), and $>$9 mm (greater than the diameter of open forceps). The total number of polyps in each size category is also assessed. The two gastroenterologists' assessments will be compared for interobserver variability and when indicated, discrepancies will be resolved by consensus review. The tattoo identification will also be reviewed to ensure tattoos are correctly identified by both assessors.

To understand the normal diet of the FAP participants, their baseline nutrient intake and the impact of the interventions on their diet, telephone interviews are undertaken by research dietitians to record all food and drink consumed by participants over the previous 24 h. The 24 h dietary recalls are undertaken 3–4 days after baseline colonoscopy, and during weeks 12 and 38 of the study. Information obtained during the phone interviews is entered directly into FoodWorks® (Version 8.0, Xyris, Queensland) by the dietitian to enable calculation of individual average daily macronutrient intakes.

Faecal samples are collected from a subset of participants recruited at RMH to determine the effects of HAMSB consumption on faecal SCFA concentrations and other faecal measures. All bowel motions passed over a 24-h period are collected on five occasions: after their screening visit in week 0 and during weeks 4, 26, 30 and 52. Collections are made at least 24 h prior to participants preparing for their colonoscopy in weeks 0, 26 and 52. They are instructed to consume their normal diets together with the appropriate starch supplement for 24 h prior to and during faecal collections. Participants are provided portable freezers, consumables and detailed instructions to ensure samples are promptly frozen after collection and delivered frozen to the hospital prior to their scopes. Samples are stored between −18 to −20 °C at the RMH and delivered in batches to CSIRO where they are stored at −80 °C until analysis. All bowel motions passed during each 24-h collection will be thawed, homogenised, weighed, subsampled and analysed for unesterified SCFA and pH as described by Watson et al. [20]. Total SCFA will be measured as described previously [6] with the exception that hydrolysis of esterified butyrate will be undertaken as described [7]. Assay controls for free and total SCFA analyses of starch and faeces are analysed approximately every ten sample injections to ensure proper analyser function. Aliquots of samples with known high, medium and low SCFA concentration were prepared, stored at −80 °C and are used periodically during the analyses as calibration controls.

Additional blood samples are collected for measurement of colorectal cancer protein biomarkers, folate, vitamin D determinations and for immune cell studies. Blood is collected into EDTA tubes for plasma for CRC protein biomarkers and processed using a double spin method as described by Fung et al. [21]. Blood is also collected into serum gel tubes for CRC protein biomarkers, vitamin D and folate measurements. After clotting for 30 min at room temperature these samples are centrifuged, decanted and batched stored at −20 °C for up to 1 month, then transferred to CSIRO for long term storage at −80 °C.

The acceptability of the starches and any unanticipated side effects not reported as adverse events will be assessed using the modified gastrointestinal quality of life index (GIQLI) [7]. The GIQLI is a validated tool requiring 1–5 scaled responses (from "all of the time" to "never") to 17 questions. The questionnaire is completed by participants on 6 occasions: before the start of the study (week 0); twice during each of the 26-week interventions (weeks 12 and 26; and 38 and 52); and at the end of the washout period (week 78).

2.1.6. Proposed statistical analyses

Descriptive statistics will be calculated for all outcome measures. All analyses will use the intention to treat population. The primary outcomes will be analysed using generalized linear mixed models for count data which allow for a range of distributional assumptions for the response variables and also for the correlation within subjects and assessors [22]. Correction for covariates will be made for baseline (pre-period 1) polyp counts, number of polyps removed at the prior colonoscopy/scope, age and surgery type. The data will be examined to identify any possible carryover effects from HAMSB ingestion into subsequent periods. The secondary outcome, GIQLI questionnaire results, will be summarised using the standard approach [7]. Other secondary outcomes will be analysed using generalized linear mixed model with an appropriate distributional form for each outcome.

Protocol deviations will be tabulated into major and minor events. Minor deviations do not carry significant ethical or administrative consequences (eg food recall undertaken on incorrect day) whereas major events are those that may affect participant safety, or the primary end measure (eg no video recording available due to equipment failure; ingestion of NSAID medication for greater than 10 days). The use of antibiotics or nonsteroidal anti-inflammatory medications for $>$10 days will be considered a major protocol deviation, and results of analyses on faeces will not be included in the data if participants consume antibiotics within a month of sample collection.

Adverse events will be reviewed using Medical Dictionary for Regulatory Activities (MedDRA) terms and will be tabulated and analysed to identify any adverse events related to treatment, surgery type, age or gender. Clinical blood values outside the normal range of the relevant participating hospital laboratory will be listed and analysed to determine if the interventions affect the incidence of these adverse events. Vital signs (heart rate, systolic/diastolic blood pressure) will be tabulated and also analysed for treatment-related adverse events.

2.1.7. Ethics and oversight

The AusFAP trial was approved by Southern Health (Melbourne, Victoria), St Vincent's Hospital (Sydney, NSW) and RBWH (Brisbane, Queensland) Human Research Ethics Committees, and site specific approval was obtained from the following sites: RMH, RBWH, St Vincent's Hospital (Sydney), Cabrini, Western Health, RCH. In addition reciprocal approval was issued from the CSIRO Health and Medical Human Research Ethics Committee. The study was registered with the Australian and New Zealand Clinical Trials Registry (https://anzctr.org.au/; ACTRN12612000804886).

A Data Safety Monitoring Committee (DSMC) was established, fully executed research collaboration agreements were signed between investigating institutions and an internal investigator management team was created to provide study oversight. The DSMC is comprised of a medical oncologist, a gastroenterologist, a colorectal surgeon and a biostatistician who are all external to the project. All have extensive clinical trial experience. A charter was prepared for the DSMC with opportunities for their input. The brief is based on the European Medicines Agency (EMEA) Guidelines,[3] the FDA's *Guidance for Clinical Trial* Sponsors, (2006),[4] *Data and Safety Monitoring Board (DSMB) Guidelines (NIH and NIDCR)*[5] and Johnson and Milewicz's (2005) presentation *Data Safety Monitoring Boards: An Education & Familiarization Module*.[6] The triggers for extraordinary DSMC meetings are if ≥ 2 cancers, or ≥ 10 SAE of any one type occur. The Management Committee will notify the DSMC if either of these situations occurs. The pre-defined increase in SAE that will prompt the DSMC to recommend to the study Management Committee to terminate the study includes, but is not limited to, a significant increase in: intercurrent operations and illnesses; abnormal blood parameters identified in SAE; the number or proportion of adenocarcinomas identified by histopathology of polyps removed during the study.

2.1.8. Results dissemination and data sharing

This study has potential to directly affect individuals with FAP and our plans to disseminate results include presentations and reports to state based FAP registries, familial cancer centres, FAP self-help organisations and to the patients of Principal Investigators where eligible individuals are recruited. As any beneficial effect of HAMSB in FAP may also have potential to translate to lower frequency of sporadic CRC in the community, we will disseminate results by professional presentations and publication in peer-reviewed journal articles.

It is anticipated that access to de-identified individual participant data underlying the published results will be available soon after publication of the main study manuscript through mediated access to the CSIRO Data Access Repository (data.csiro.au). Use of this repository is intended for the long term storage of study data.

[3] EMEA (2005) *Guideline on Data Monitoring Committees*; available at: http://www.emea.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003635.pdf.

[4] http://www.fda.gov/downloads/RegulatoryInformation/Guidances/ucm127073.pdf.

[5] http://www.nidcr.nih.gov/Research/ToolsforResearchers/Toolkit/DSMBGuidelines.htm.

[6] Downloaded from http://people.musc.edu/~elg26/talks/SAEReportingandDSMBs.pdf.

3. Discussion

Butyrate is normally produced in the large bowel during the microbial fermentation of dietary fibre and may protect from colon cancer by regulating host molecular mechanisms underlying CRC, including modulating mucosal gene expression and the host immune response. Butyrate is an histone deacetylase (HDAC) inhibitor [23] and an agonist of several G-protein coupled receptors (GPCR)s [24]. Via these functions it has been shown to modulate intracellular signalling cascades that promote apoptotic and anti-inflammatory pathways and protect against tumorigenesis [25]. In animal models, butyrate has been shown to attenuate the production of inflammatory cytokines, induces differentiation of T_{reg} cells in the colonic mucosa [26] and promotes the anti-inflammatory properties of macrophages and dendritic cells. In cell culture, butyrate stimulates apoptosis of oncogenic cells [9], promotes colonocyte differentiation and inhibit growths of CRC cells [23]. Butyrate also suppresses tumour growth by inhibiting aerobic glycolysis and reversing the Warburg effect, inhibiting the growth of cancerous cells while sparing normal cells [23]. Butyrate regulates mucosal health, defence and repair through direct effects on large bowel mucosa by activating macrophage and goblet cell functions [27].

These effects may also translate to humans. Epidemiological studies have implicated factors including diets low in dietary fibre and high in processed meats as major risk factors for the development of CRC [3]. High dietary intake of red meat has been shown to induce the expression of oncogenic micro RNAs (miRNA), including those of the miR17–92 cluster and miR21, in rectal mucosal tissue of healthy human volunteers. Supplementation of these diets with the butyrate-elevating fibre HAMSB was shown to normalise levels of these oncogenic miRNAs [12].

Raising large bowel butyrate concentration has the potential to improve colonic health, particularly in individuals consuming a diet low in fermentable fibre and those unable to ferment types of resistant starch [28]. HAMSB increases the amount of this SCFA in the colon of healthy individuals [7] where microbial enzymes release the esterified butyrate for mucosal absorption or use by colonocytes. HAMSB has also been shown to significantly increase the faecal concentration of butyrate in FAP with IRA and IPAA, indicating their reduced colonic microflora remain capable of releasing bound acid from the starch base.

The present study will test our hypothesis that HAMSB will deliver butyrate to the large bowel of FAP participants and as a consequence, reduce initiation and/or growth of polyps in these individuals. AusFAP is the first clinical trial to evaluate the effects of a cost-effective food supplement that delivers significant quantities of butyrate to the colon, and the study has potential to provide insights into the interaction between diet and host metabolism in the development of CRC. Positive results may indicate potential benefits from long-term HAMSB dietary supplementation to reduce the risk of CRC development in the FAP population, and may extrapolate to sporadic CRC in the wider community where low dietary fibre intake is a major factor contributing to the high incidence of this disease.

Contributions

Finlay Macrae: funding acquisition, conceptualization and methodology, supervision, investigation, review and editing. **Alex Boussioutas**: funding acquisition, conceptualization, investigation, review and editing. **Trevor Lockett**: funding acquisition, conceptualization, supervision, review and editing. **Julie Clarke**: funding acquisition, project administration, data curation, preparing original draft, review and editing. **Brooke Flanders:** project administration, investigation, data curation. **Karen Harrap**: software development, data management, review and editing. **Ian Saunders**: statistical design, software development, review and editing. **Patrick Lynch:** ideas, conceptualization and methodology. **Mark Appleyard**: investigation, review and editing. **Allan Spigelmam**: investigation, review and editing. **Don Cameron:** investigation, review and editing.

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References

[1] J. Ferlay, M. Colombet, I. Soerjomataram, C. Mathers, D.M. Parkin, M. Pineros, A. Znaor, F. Bray, Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods, Int. J. Cancer 144 (2019) 1941–1953.

[2] D.C. Whiteman, P.M. Webb, A.C. Green, R.E. Neale, L. Fritschi, C.J. Bain, D. M. Parkin, L.F. Wilson, C.M. Olsen, C.M. Nagle, N. Pandeya, S.J. Jordan, A. Antonsson, B.J. Kendall, M.C. Hughes, T.I. Ibiebele, K. Miura, S. Peters, R. N. Carey, Cancers in Australia in 2010 attributable to modifiable factors: summary and conclusions, Aust. N. Z. J. Publ. Health 39 (2015) 477–484.

[3] World Cancer Research Fund International/American Institute for Cancer Research, Continuous Update Project Report: Diet, Nutrition, Physical Activity and Colorectal Cancer, 2017. Available at: wcrf.org/colorectal-cancer-2017.

[4] S.J. O'Keefe, Diet, microorganisms and their metabolites, and colon cancer, Nat. Rev. Gastroenterol. Hepatol. (2016).

[5] G. Annison, R.J. Illman, D.L. Topping, Acetylated, propionylated or butyrylated starches raise large bowel short-chain fatty acids preferentially when fed to rats, J. Nutr. 133 (2003) 3523–3528.

[6] J.M. Clarke, A.R. Bird, D.L. Topping, L. Cobiac, Excretion of starch and esterified short chain fatty acids by ileostomists after the ingestion of acylated starches, Am. J. Clin. Nutr. 86 (2007) 1146–1151.

[7] J.M. Clarke, D.L. Topping, C.T. Christophersen, A.R. Bird, K. Lange, I. Saunders, L. Cobiac, Butyrate esterified to starch is released in the human gastrointestinal tract, Am. J. Clin. Nutr. 94 (2011) 1276–1283.

[8] J.M. Clarke, G.P. Young, D.L. Topping, A.R. Bird, L. Cobiac, B.L. Scherer, J. G. Winkler, T.J. Lockett, Butyrate delivered by butyrylated starch increases distal colonic epithelial apoptosis in carcinogen-treated rats, Carcinogenesis 33 (2012) 197–202.

[9] S. Toden, T.J. Lockett, D.L. Topping, B.L. Scherer, E.J. Watson, J.G. Southwood, J. M. Clarke, Butyrylated starch affects colorectal cancer markers beneficially and dose-dependently in genotoxin-treated rats, Cancer Biol. Ther. 15 (2014) 1515–1523.

[10] J.M. Clarke, D.L. Topping, A.R. Bird, G.P. Young, L. Cobiac, Effects of high amylose maize starch and butyrylated high amylose maize starch on azoxymethane-induced intestinal cancer in rats, Carcinogenesis 29 (2008) 2190–2194.

[11] K.J. Humphreys, M.A. Conlon, G.P. Young, D.L. Topping, Y. Hu, J.M. Winter, A. R. Bird, L. Cobiac, N.A. Kennedy, M.Z. Michael, R.K. Le Leu, Dietary manipulation of oncogenic MicroRNA expression in human rectal mucosa: a randomized trial, Cancer Prev. Res. 7 (2014) 786–795.

[12] R.K. Le Leu, J.M. Winter, C.T. Christophersen, G.P. Young, K.J. Humphreys, Y. Hu, S.W. Gratz, R.B. Miller, D.L. Topping, A.R. Bird, M.A. Conlon, Butyrylated starch intake can prevent red meat-induced O6-methyl-2-deoxyguanosine adducts in human rectal tissue: a randomised clinical trial, Br. J. Nutr. 114 (2015) 220–230.

[13] W.E. Roediger, Utilization of nutrients by isolated epithelial cells of the rat colon, Gastroenterology 83 (1982) 424–429.

[14] J. Serpa, F. Caiado, T. Carvalho, C. Torre, L.G. Goncalves, C. Casalou, P. Lamosa, M. Rodrigues, Z. Zhu, E.W. Lam, S. Dias, Butyrate rich colonic microenvironment is a relevant selection factor for metabolically adapted tumour cells, J. Biol. Chem. 285 (2010) 39211–39223.

[15] H.Y. Yoo, S.Y. Park, S.Y. Chang, S.H. Kim, Regulation of butyrate-induced resistance through AMPK signaling pathway in human colon cancer cells, Biomedicines 9 (2021).

[16] P.M. Lynch, Chemoprevention with special reference to inherited colorectal cancer, Fam. Cancer 7 (2008) 59–64.

[17] J. Clarke, A. Boussioutas, T. Lockett, F. Macrae, Release of dietary esterified butyrate in participants with familial adenomatous polyposis, J. Gastroenterol. Hepatol. 27 (2012), 125-125.

[18] S.P.A. International, Starch institute, Denmark, ISI 14-2e Determination of Acetyl Conten (1999) t, http://www.starch.dk/isi/methods/14acet.htm.

[19] H. Ishikawa, K. Wakabayashi, S. Suzuki, M. Mutoh, K. Hirata, T. Nakamura, I. Takeyama, A. Kawano, N. Gondo, T. Abe, S. Tokudome, C. Goto, N. Matsuura, T. Sakai, Preventive effects of low-dose aspirin on colorectal adenoma growth in patients with familial adenomatous polyposis: double-blind, randomized clinical trial, Cancer Med. 2 (2013) 50–56.

[20] E.J. Watson, J. Giles, B.L. Scherer, P. Blatchford, Human faecal collection methods demonstrate a bias in microbiome composition by cell wall structure, Sci. Rep. 9 (2019), 16831.

[21] K.Y.C. Fung, L. Purins, I.K. Priebe, C. Pompeia, G.V. Brierley, B. Tabor, T. Lockett, P. Gibbs, J. Tie, P. McMurrick, J. Moore, A. Ruszkiewicz, A. Burgess, E. Nice, L. J. Cosgrove, Analysis of 32 blood-based protein biomarkers for their potential to diagnose colorectal cancer, J. Mol. Biomarkers Diagn. (2014) S6.

[22] W.W. Stroup, Generalized Linear Mixed Models: Modern Concepts, Methods and Applications, CRC Press, 2012.

[23] J.M. Mariadason, HDACs and HDAC inhibitors in colon cancer, Epigenetics 3 (2008) 28–37.

[24] J.K. Tan, C. McKenzie, E. Mariño, L. Macia, C.R. Mackay, Metabolite-sensing G protein-coupled receptors-facilitators of diet-related immune regulation, Annu. Rev. Immunol. 35 (2017) 371–402.

[25] N. Singh, A. Gurav, S. Sivaprakasam, E. Brady, R. Padia, H. Shi, M. Thangaraju, P. D. Prasad, S. Manicassamy, D.H. Munn, J.R. Lee, S. Offermanns, V. Ganapathy, Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis, Immunity 40 (2014) 128–139.

[26] Y. Furusawa, Y. Obata, S. Fukuda, T.A. Endo, G. Nakato, D. Takahashi, Y. Nakanishi, C. Uetake, K. Kato, T. Kato, M. Takahashi, N.N. Fukuda, S. Murakami, E. Miyauchi, S. Hino, K. Atarashi, S. Onawa, Y. Fujimura, T. Lockett, J.M. Clarke, D.L. Topping, M. Tomita, S. Hori, O. Ohara, T. Morita, H. Koseki, J. Kikuchi, K. Honda, K. Hase, H. Ohno, Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells, Nature 504 (2013) 446–450.

[27] L. Liang, L. Liu, W. Zhou, C. Yang, G. Mai, H. Li, Y. Chen, Gut microbiota-derived butyrate regulates gut mucus barrier repair by activating the macrophage/WNT/ERK signaling pathway, Clin. Sci. 136 (2022) 291–307.

[28] J.H. Cummings, E.R. Beatty, S.M. Kingman, S.A. Bingham, H.N. Englyst, Digestion and physiological properties of resistant starch in the human large bowel, Br. J. Nutr. 75 (1996) 733–747.