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RESEARCH ARTICLES

A food-based approach that targets interleukin-6, a key regulator of chronic intestinal inflammation and colon carcinogenesis

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Abstract

Studies have shown a causal link between high-calorie diet (HCD) and colon cancer. However, molecular mechanisms are not fully elucidated. To understand etiology of HCD-induced colon carcinogenesis, we screened 10 pathways linked to elevated colonic cell proliferation and chronic inflammation in an HCD-consuming human-relevant pig model. We observed elevated colonic mucosal interleukin-6 (IL-6) expression in HCD-consuming pigs compared to standard diet controls (SD, $P=.04$), and IL-6 strongly correlated with Ki-67 proliferative index and zone, early biomarkers of colon cancer risk ($r=0.604$ and 0.743 and $P=.017$ and .002, respectively). Liquid chromatography–tandem mass spectrometry-based proteomic analysis and Ingenuity Pathway Analysis showed that HCD consumption altered IL-6 signaling pathway proteins (PI3KR4, IL-1α, Mapk10, Akt3, PIK3CG, PIK3R5, Map2k2). Furthermore, these proteins also correlated with Ki-67 proliferative index/zone. Anti-IL-6 therapeutics are available for treating colon cancer; however, they are expensive and induce negative side effects. Thus, whole foods could be a better way to combat low-grade chronic colonic inflammation and colon cancer. Whole plant foods have been shown to decrease chronic diseases due to the potential of anti-inflammatory dietary compounds acting synergistically. We observed that supplementation of HCD with anthocyanin-containing purple-fleshed potatoes (10% w/w), even after baking, suppressed HCD-induced IL-6 expression ($P=.03$) and the IL-6-related proteins IL-1α and Map2k1 ($P\leq.1$). Our results highlight the importance of IL-6 signaling in diet-linked induction/prevention of colonic inflammation/cancer and demonstrate the potential of a food-based approach to target IL-6 signaling.

Keywords: Colonic inflammation; Colon cancer; Interleukin-6; High-calorie diet; Purple-fleshed potatoes; Pig model

1. Introduction

Alongside the development of technology came a shift in our diet [1]. Westernized populations moved from hunter–gatherer lifestyles with minimal food processing to novel foods such as dairy products, cereals, refined sugars and fatty meats [1], common staples of the current Western diet. Furthermore, foods became easily accessible at low cost and increased convenience. Marked by high fat and high sugar, the Western diet is leading to a greater caloric intake in the society. This high-calorie diet (HCD) coupled with a sedentary lifestyle is linked to multiple diseases, including obesity, type 2 diabetes, cardiovascular disease and certain cancers such as colon cancer [2,3].

Colon cancer is the second leading cause of cancer-related deaths in the United States. Approximately 5% (1 in 20) of Americans will be diagnosed with colon cancer in their lifetime [4]. Consumption of an HCD has shown to increase the risk for colon cancer in a human model [5–7]. Recent animal studies also suggest a causal link between HCD and increased colon cancer risk [8–13]. Eighteen months of consumption of the Western diet induced colonic tumors in normal C57Bl/6 mice [13] in the absence of any carcinogen. Furthermore, a recent study by Erdelyi et al. [14] showed that the high-fat Western diet negatively impacted colonic lipid metabolism, oxidative stress, and immune responses in C57Bl/6 mice. Colonic inflammation plays an important role in elevating the risk for colon cancer with the implication of multiple pathways, including the interleukin-6 (IL-6) signaling pathway [15–17]. The association of IL-6 signaling with colon cancer was clearly demonstrated in a recent study by Day et al. [10]. These researchers found elevated IL-6

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expression in colonic polyps of $Apc^{Min/+}$ mice fed an HCD compared to those fed a standard diet (SD), suggesting that HCD can drive the increased production of proinflammatory cytokines, such as IL-6, thus elevating the risk for colon cancer.

IL-6 is a proinflammatory cytokine released by myeloid cells in various tissues crucial for immune response, cell survival, apoptosis and proliferation [18–21]. IL-6 also regulates the proliferation of intestinal epithelial cells [22]. Recent studies propose a link between chronic inflammatory diseases (*e.g.*, irritable bowel syndrome and colon cancer) and IL-6 signaling [16,18,20,23–25]. A study in C57BL/6 mice found higher IL-6 mRNA and protein expression in the dextran sodium sulfate and azoxymethane (DSS/AOM)-induced colon cancer tumors than surrounding normal colon tissue, suggesting that IL-6 may be responsible for enhanced colon carcinogenesis [26]. IL-6, when bound to its receptor IL-6R, leads to downstream activation of the JAK/STAT3 pathway, inducing expression of genes important in elevation of proliferation and suppression of apoptosis [18]. Recent work by Grivennikov et al. [18] in IL-6 knockout mice reported a decrease in Ki-67-expressing colon crypt cells, an early biomarker for colon cancer. Other studies have added evidence to propose that IL-6 acts in colon cancer by increasing proliferation (reviewed in [27]). Taken together, these studies suggest that IL-6 provides resistance to apoptosis and provides a conducive environment for increased cell proliferation, ultimately leading to enhanced cell growth and survival. Augmented IL-6 and its downstream signaling pathways may provide a proinflammatory milieu favorable for colon cancer development.

Currently, various anti-IL-6 therapeutics are being used or are in clinical trials for multiple diseases and cancers including colon cancer, multiple myeloma, prostate cancer, and Castleman disease [17,28]. The therapeutics available for colon cancer treatment currently target inhibition of the IL-6/STAT3 signaling pathway with anti-IL-6 receptor antibodies, soluble gp130Fc and small molecule JAK inhibitors [17]. The use of anti-IL-6 receptor antibodies has been shown to suppress the growth of colon tumors and protect against colon carcinogenesis in DSS/AOM-induced colon carcinogenesis in C57BL/6 mice [26]. While these therapeutics provide a potential therapy for cancer patients, therapeutics can be costly and include an array of negative side effects or even lead to drug tolerance [17]. Therefore, there is a growing interest in alternative therapeutics that could alleviate the cost and side effects patients face, reducing the stress of the already existing physical and psychological burden of disease.

Bioactive compounds from plants, including anthocyanins and phenolic acids, are linked to a reduced risk for a variety of cancers, including colon cancer [29–39]. However, individual phytochemicals have been shown to have proinflammatory and procancerous effects in high doses alone (reviewed in [40]). Plant foods have been illustrated in epidemiological studies and other research to hold a potential for disease prevention as different dietary ingredients can work synergistically to enhance the activity of a single compound, providing a better explanation for the benefits of whole foods observed in epidemiological studies [41–43].

Color-fleshed potatoes contain a variety of secondary metabolites, including polyphenols. Specifically, purple-fleshed potatoes are rich in phenolic acids and anthocyanins. Our previous studies linked the anti-colon-cancer properties of color-fleshed potatoes to their bioactive compounds [44–46]. Consumption of color-fleshed potatoes has been on the rise in the past 10 years, likely due to their putative health benefits. While studies on individual compounds and their anticancer effects have been performed, the benefits of plant foods such as anthocyanin-containing purple-fleshed potatoes, rich in anthocyanins, against colonic inflammation/cancer in humans or in a human-relevant model are lacking.

Developing an appropriate model for *in vivo* studies is crucial to best understand and extrapolate the data to human models. While mice are popular models for studying a variety of diseases, the anatomical and physiological differences between rodents and humans are significant. In addition, wide differences in dietary patterns exist between the two groups. The human is an omnivore, whereas rats and mice were originally granivores. Other models exist, including cats/dogs, but these models have differing diet and meal patterns than humans. Primates are rarely used in food intake studies due to their expense and scarcity [47]. In contrast, the pig is an excellent model to study the nutrition and food intake in humans. This study used a pig model because it is experimentally tractable and it is a clinically relevant model of the human gastrointestinal tract [47–49].

Using a human-relevant porcine model, we investigated the effect of HCD on colonic inflammation. We screened a panel of inflammatory biomarkers involved in colonic inflammation/cancer and identified the IL-6 signaling as the prominently altered pathway using quantitative polymerase chain reaction (qPCR) and proteomics analysis. Proteins in the IL-6 signaling pathway as well as IL-6 significantly correlated with Ki-67 proliferative index and zone, early biomarkers of colon cancer. Thus, our data revealed the role of IL-6 and its signaling pathway in enhancing colonic proliferation in colon cancer development in a human-relevant model. We further evaluated if dietary intervention of purple-fleshed potatoes could alleviate HCD-induced colonic inflammation. We witnessed suppression in IL-6 expression with the supplementation of only 10% w/w purple-fleshed potatoes, even after baking, in HCD-consuming pigs. Our study suggests staple crops that contain anthocyanins should be further as potential dietary interventions in the prevention and treatment of gastrointestinal inflammation/cancers.

2. Experimental section

2.1. Diet-induced inflammation

Male pigs (6 weeks old) were obtained from Smithfield Premium Genetics (Rose Hill, NC, USA) and housed individually in indoor pens at the North Carolina State University Swine Educational Unit (Clayton, NC, USA). The animals were allocated into different treatment groups by body weight so that mean initial body weight was similar among the treatment groups (N=8 animals/treatment).

2.2. Experimental diets

Animals were provided with one of the following diets: a SD (~5% fat), an HCD (17% added dry fat and ~3%–5% endogenous fat), or HCD supplemented with 10% of purple-fleshed potato (raw or baked). Purple-fleshed potatoes (Purple Majesty) were grown at Black Gold Farms (Pearsall, TX, USA). Baking of potatoes was done at Worldwide Food (Burley, ID, USA). Raw and baked potatoes were freeze-dried at Vandrunen Farms, IL, USA, prior to incorporation in the diet. White corn and dry fat were used as a major energy source, and soybean meal was the major protein source. Ratios between corn and soybean meal were adjusted to match energy and protein contents among diets. White corn was used to prevent carotenoids from yellow corn affecting the study. Composition of all the diets is presented in Table A1. Pigs consumed the experimental diets for 13 weeks — the feed and drinking water were provided *ad libitum*.

2.3. Colon tissue collection

The animals were euthanized at the end of the study using a captive bolt followed by exsanguination. The distal colon was resected and was cleaned with RNAse-free phosphate-buffered saline, and the mucosa was scraped using a glass slide into an RNAse-free tube. The tube was snap frozen in liquid nitrogen and later transferred to −80°C.

2.4. Inflammatory markers

qPCR analysis was used to measure gene expression of proinflammatory markers in the distal colon mucosa. Total RNA was extracted using the Phenol-free total RNA purification kit (Amresco, Solon, OH, USA), and cDNA synthesis was carried out using the qScript cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD, USA) according to the manufacturer's instructions. qPCR was performed using qPCR Sybr green supermix (Quanta Biosciences) on a Roche Lightcycler 96 machine, and mRNA expression of genes was calculated using the Roche Lightcycler Software (Roche Diagnostics, Indianapolis, IN, USA). Primers used for the study are presented in Table A2.

2.5. Cell proliferation

Data on Ki-67 proliferative index and zone in the distal colon, early biomarkers of colon cancer, were used for correlations. To measure Ki-67 proliferative index and zone, only U-shaped, longitudinally cut crypts with open lamina and base touching the muscularis mucosa were selected for assessing the yield and distribution of labeled cells along the crypt. Twenty to 25 crypts per animal were counted. The total number of immunoreactive nuclei and the total number of nuclei per crypt column were counted. The proliferation index was defined as the percentage labeled cells of the total number of crypt column cells. To assess the distribution of the labeled cells, the proliferative zone was calculated as the number of cells from the base to the most upper labeled cell divided by the total number of cells in the column multiplied by 100.

2.6. Proteomics

Distal colon mucosal protein was extracted using the complete mammalian proteome extraction kit (Millipore, Billerica, MA, USA). After protein extraction, 100 μg each of protein samples was reduced, alkylated and double-trypsin digested. Dried peptides were reconstituted in 0.1% formic acid, and 20 μg of tryptic peptides was injected onto a Polaris 2-mm × 100-mm c-18 RP column using ultra high-performance liquid chromatography (LC) at a flow rate of 0.4 ml/min. All the samples were analyzed in triplicates on a high-flow LC/Agilent Jet Stream Dual Ion Funnel Quadrupole Time-of-Flight (Q-TOF) mass spectrometer using a 30-min gradient. The LC–tandem mass spectrometry (MS/MS) data were searched against NCBInr *Sus scrofa* database using Spectrum Mill bioinformatics tool. At the 1% false discovery rate (FDR) level, altogether about 4000 unique porcine proteins were identified in the preliminary analysis. Pathway analysis was performed using Ingenuity Pathway Analysis (IPA) application from Ingenuity Systems (Redwood City, CA, USA). The data set containing accession numbers (human homologs of porcine proteins) and corresponding expression values of the differentially altered proteins was uploaded into the application, and the accession numbers were mapped using the IPA software. The IPA software identified the pathways from the IPA library that are most significant to the data set. Fisher's Exact Test was used to calculate the *P* value, determining the probability that each canonical pathway assigned to this data set was not due to chance alone.

2.7. Statistical analysis

Completely randomized block design was used in this study. Individual pigs were the experimental unit. Comparisons between two groups (SD and HCD) were performed using Mann–Whitney *U* test using IBM SPSS v21 software (IBM, Armonk, NY, USA). The statistical procedure used for analysis of the data of four groups (SD, HCD, PR and PB) was PROC MIXED (mixed model) using SAS software (SAS Institute, Cary, NC, USA). The results were expressed as means ± S.E. for each treatment. Spearman correlations were run using IBM SPSS v21 software.

3. Results

3.1. Pathway screening

We used a pig model to test HCD-induced inflammatory pathways, as it closely resembles human gastrointestinal anatomy and physiology. To understand alterations in inflammatory pathways caused by HCD,

Table 1
Inflammatory proteins screened using qPCR in the distal colon mucosa

Pathway	Biomarker gene	Relative expression		*P* value
		SD	HCD	
Inflammasome	Interleukin-1β	1.29±0.25	1.10±0.19	.867
	Interleukin-18	1.27±0.19	1.10±0.40	.232
Interleukin-6 signaling	**Interleukin-6**	**0.52±0.20**	**1.63±0.52**	**.040**
Gut permeability	Occludin	1.05±0.14	1.11±0.18	.694
	Tight junction protein-1	0.94±0.07	1.00±0.19	.613
	Proteasome beta-defensin-2	0.08±0.08	0.05±0.03	.426
	Angiotensin-converting enzyme 2	1.84±0.63	1.12±0.25	.336
TLR3 signaling	Chemokine ligand 5	1.85±0.25	1.43±0.47	.397
	Lipocalin-2	1.43±0.33	1.43±0.40	.613
	Toll-like receptor-3	1.07±0.13	1.14±0.29	.463
	Enolase-1	1.17±0.16	1.19±0.17	.482
Glycolysis	Phosphofructokinase	2.91±1.00	2.97±0.69	.779
	Phosphoglycerate kinase 1	1.12±0.15	1.12±0.08	.244
	Phosphoglucose isomerase	1.60±0.12	2.14±0.33	.694
C-Jun./MELK pathway	Maternal embryonic leucine zipper kinase	1.24±0.24	1.20±0.10	.694
Rho proteins	Ras homolog family member A	1.10±0.13	1.07±0.15	.867
	Ras-related C3 botulinium toxin substrate-1	1.59±0.17	1.82±0.24	.779
Netrin signaling pathway	Netrin-1	1.51±0.17	1.24±0.20	.463
	UNC-5 Homolog B	1.59±0.17	1.46±0.34	.336
	Binding immunoglobulin protein	1.20±0.21	1.20±0.06	.463
ER stress	DNA damage-inducible transcript 3	0.79±0.13	0.73±0.22	.613
	Interferon-γ	1.85±0.59	1.18±0.20	.336
NF-κB pathway	Tumor necrosis factor-α	0.65±0.15	1.58±0.54	.073
	v-rel avian reticuloendotheliosis viral oncogene homolog A	0.54±0.29	0.17±0.04	.351

The relative expression of the genes is reported for SD- and HCD-consuming animals as mean ± SE for six to seven animals in each group. Comparisons between the two groups were performed using Mann–Whitney *U* test.

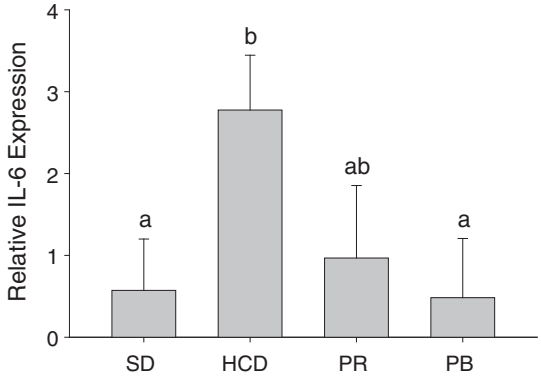

Fig. 1. IL-6 expression in the distal colon mucosa of pigs consuming SD, HCD or HCD supplemented with 10% raw or baked purple-fleshed potatoes (PR and PB, respectively) was measured using qPCR. Results were expressed as mean ± SE for six to seven animals per group. Means that differ by letter (a, b) differ $P \leq .03$.

we screened 10 different pathways including 23 genes (Table 1) known for their involvement in inflammation. These pathways have been previously linked to inflammatory disorders and colon cancer risk.

Previous studies have suggested that elevated IL-6 expression plays a critical role in multiple chronic inflammatory diseases including Crohn's disease, ulcerative colitis and colon cancer [18,20,23,24,50]. Through our pathway screening, we found approximately a threefold increase in IL-6 expression in distal colon mucosa with HCD supplementation compared to SD (Fig. 1, Table 1).

Spearman correlations were performed to understand the potential link between inflammation and proliferation. The increased expression of IL-6 correlated with Ki-67 proliferative index ($r=0.604$, $P=.017$) and zone ($r=0.743$, $P=.002$; Table 2), indices of colon crypt cell proliferation and early biomarkers of colon cancer. Strong correlations suggest that the colon crypt was undergoing elevated proliferative signaling possibly due to elevated IL-6 linked inflammation in HCD-consuming animals.

3.2. Proteomics analysis of HCD effect on IL-6 signaling

We performed proteomics analysis of the distal colon mucosa using LC–MS/MS in both SD- and HCD-consuming animals to investigate the effects of dietary alteration on IL-6 signaling. Our proteomic analysis identified the IL-6 signaling pathway to be altered by HCD ($P=.06$ in IPA), thus confirming our previous findings of alterations in mRNA expression. Through proteomic analysis, we identified several proteins upstream or downstream of IL-6 altered by HCD. Seven proteins in the IL-6 signaling pathway were altered (Table 3), with four proteins up-regulated by HCD (PI3KR4, IL1α, Mapk10, Akt3) and three protein levels down-regulated by HCD (PIK3CG, PIK3R5, Map2k2). Of the seven, six proteins were either completely knocked down or only induced due to HCD consumption. IPA data revealed that IL-6 potentially up-regulates the PI3K/Akt pathway in the human-relevant pig model of HCD-induced inflammation. Proteins altered by HCD in this pathway (PIK3CG, MAPK10, PIK3R5 and Akt3) significantly correlated with Ki-67 proliferative index and/or zone (Table 2), suggesting a link between IL-6 signaling and enhanced colonic proliferation. The correlations suggest the involvement of IL-6 in increasing colon cancer risk.

Table 2
Correlations of IL-6 and proteins in the IL-6 signaling pathway identified using proteomics of pig distal colon mucosa of HCD consuming animals, with Ki-67 proliferative index and zone

	Ki-67 proliferative index	Ki-67 proliferative zone
IL-6	0.604±0.017 *	0.743±0.002 **
PIK3CG	−0.653±0.021 *	−0.586±0.045 *
MAPK10	0.390±0.210	0.686±0.014 *
PIK3R5	−0.566±0.055	−0.703±0.011 *
Akt3	0.811±0.001 **	0.491±0.105

Correlations are reported as Spearman correlation coefficient ± P value.
* Represents correlations $P \leq .05$.
** Represents correlations $P \leq .01$.

Table 3
Relative abundances of proteins in IL-6 signaling pathway analyzed using LC–MS/MS

Protein	SD	HCD	P value
PI3KR4	$7.23\times10^5\pm1.23\times10^5$	$1.434\times10^6\pm2.07\times10^5$	.065
IL-1α	0±0	$2.40\times10^6\pm8.51\times10^5$	.061
MAPK10	0±0	$1.31\times10^6\pm3.22\times10^5$	.015 *
PIK3CG	$2.58\times10^6\pm4.90\times10^5$	0±0	.002 *
PIK3R5	$3.74\times10^6\pm1.52\times10^6$	0±0	.061
Akt3	0±0	$4.31\times10^6\pm3.77\times10^6$	.015 *
Map2k2	$2.97\times10^5\pm1.33\times10^5$	0±0	.061
Map2k1	0±0	$3.99\times10^5\pm3.15\times10^5$	.455

The relative abundances are reported for SD- and HCD-consuming animals as mean ± SE for six to seven animals per group. Comparisons between the two groups were performed using Mann–Whitney U test.
* Represents significant difference compared to SD, $P \leq .05$.

3.3. Impact of dietary intervention on inflammation markers

To assess the effects of dietary interventions in the prevention of HCD-induced inflammation, distal colon mucosa from pigs consuming HCD supplemented with either raw or baked purple-fleshed potatoes (10% w/w) was evaluated for IL-6 expression using qPCR (Fig. 1). Although not significant, raw purple-fleshed potatoes suppressed HCD-induced IL-6 expression by approximately threefold ($P=.12$). Meanwhile, purple-fleshed baked potatoes significantly suppressed HCD-induced IL-6 expression by almost six times, bringing it to levels similar to SD-consuming animals ($P=.03$). Proteomics analysis revealed alterations in two proteins involved in IL-6 signaling with a potato-supplemented HCD diet. IL-1α was significantly up-regulated by HCD compared to SD ($P=.01$), but significantly reduced by raw purple-fleshed potato supplementation ($P=.01$) and followed a trend of down-regulation with purple-fleshed baked potato supplementation ($P=.08$). A second protein, Map2k1, involved in IL-6 signaling *via* its roles in proliferation, differentiation and transcription regulation, followed the same trends as IL-1α with both purple-fleshed raw and baked potato supplementation exhibiting a trend to lower HCD-induced elevation (Table 4). Potatoes are typically consumed after processing, and our data show that processed purple-fleshed potatoes exhibited anti-inflammatory benefits and prevented HCD-induced inflammation *in vivo*. Overall, as expected, potato supplementation could prevent inflammatory signaling caused by HCD, likely due to their high anthocyanin and bioactive compound levels. Thus, our data provide evidence of the beneficial effects of a whole food against inflammation markers and potential colon cancer risk.

4. Discussion

Although evidence links HCD to colonic inflammation and colon cancer, there have been no studies on this aspect in a human-relevant

Table 4
Relative abundances of proteins in IL-6 signaling pathway analyzed using LC–MS/MS

Protein	SD	HCD	PR	PB
IL-1α	$0\pm6.00\times10^5$ **	$2.4\times10^6\pm6.00\times10^5$	$0\pm6.00\times10^5$ **	$8.46\times10^5\pm6.00\times10^5$ *
Map2k1	$0\pm1.57\times10^5$ *	$3.99\times10^5\pm1.57\times10^5$	$0\pm1.57\times10^5$ *	$0\pm1.57\times10^5$ *

The relative abundance are reported for animals consuming SD, HCD or HCD supplemented with 10% raw or baked purple-fleshed potatoes (PR and PB, respectively) as mean ± SE (distributed across treatments) for six to seven animals per group. Comparisons between the groups were made using mixed model using SAS software.
* Represents P value ≤.1 compared to HCD.
** Represents P value ≤.05 compared to HCD.

porcine model. Previous research focused on HCD-induced inflammation in mouse models. Further, studies that evaluated dietary interventions of bioactive compound (anthocyanin) containing foods often evaluate extracts rather than whole foods. Therefore, the purpose of this study was to use a human-relevant porcine model to investigate the anti-inflammatory role of purple-fleshed potatoes as a dietary intervention through understanding the mechanism of HCD-induced inflammation and its link to colon cancer.

Recent studies have shown that HCD increases the incidence of colon cancer by providing a conducive environment for colonocyte proliferation [8]. While data are available in mouse models, human studies are limited to epidemiological studies, as systematic studies cannot be completed in humans due to ethical and logistical concerns. Although mouse studies provide insight of the effects of HCD on inflammation and cancer, they are difficult to translate to humans. Therefore, we utilized a human-relevant porcine model, as it is most similar to humans in gastrointestinal anatomy and physiology [47–49]. A human-relevant model can provide more translatable information on the effects of HCD on colonic inflammation and its relation to colon cancer risk. This can ultimately aid to develop targeted therapies or dietary interventions.

Our model showed the greatest alterations due to HCD in the IL-6 signaling pathway. We further validated our findings in mRNA expression using proteomics analysis. We analyzed distal colon mucosa lysates to uncover the effects of HCD on protein expression. We confirmed that the IL-6 pathway was altered, as many proteins in the IL-6 signaling pathway (identified using IPA) were either elevated or suppressed by HCD (Table 3). IL-1α followed a trend of up-regulation with HCD consumption. Recent studies have shown that IL-1α is prevalent in tumor microenvironments in pancreatic cancer and hepatic cancer, suggesting that it could also play a potential role in early inflammation leading to colon cancer [51,52]. From the data obtained using IPA analysis, we believe that IL-1α activates the IL-6 pathway by binding to its receptor and causing an increase in the JNK/MAPK pathway that is linked to regulation of cell proliferation, cytokine secretion and apoptosis [53]. This is supported by alteration in the MAP kinase proteins in our proteomics data. This leads to up-regulated transcription of IL-6, supported by our qPCR analysis in distal colon mucosa tissue. The up-regulation of IL-6 further causes a signal cascade activating the pro-proliferative PI3K/Akt pathway, leading to alterations in PI3K and Akt proteins, as evidenced in proteomic analysis data (Table 3).

This evidence suggests that HCD induces an inflammatory milieu, potentially through the IL-6 pathway. However, there is evidence that alternative pathways may be activated *via* IL-6 based on our results. While we did not see a significant increase in TNF-α in this study, it has been shown that TNF-α is activated *via* IL-6 signaling [18]. HCD-induced elevation of TNF-α was not significant but numerically elevated, likely due to the duration of this study. As the pig lifespan is greater than the mice, chronic inflammation may progress slowly in pigs. However, data from our previous studies show that HCD induced elevation of systemic TNF-α in the pig model [54,55]. Our proposed mechanism of inflammation is further confirmed by our previous findings of increased oxidative stress and inflammation [56]. Furthermore, we have previously looked at the well-known inflammatory biomarker NF-κB, in which we reported a significant elevation with HCD in the distal colon [57]. Although we did not present the data, anti-inflammatory proteins IL-10 and TGF-β levels were not altered between the HCD and SD group. The previous findings along with the new findings presented here of IL-6 signaling pathway elevation support the use of the pig model to understand HCD-induced inflammation in relation to early markers of colon cancer such as proliferative zone and index.

An early risk of colon cancer can be detected using immunofluorescence staining of Ki-67, a marker of proliferation [58,59]. Our previous work has illustrated that distal colon crypt Ki-67 proliferative index and zone are increased with HCD and that it correlated with proinflammatory markers [57]. The current study showed a significant correlation of increased IL-6 expression and proteins in the IL-6 signaling pathway with increased Ki-67 colon crypt proliferation index and zone, early markers for colon cancer risk (Tables 2 and 3). Previous studies have reported decreased Ki-67 in IL-6 knockout mice [18]. IL-6 is well known to increase risk for colon cancer, and there have been multiple studies on IL-6 on how it acts as a link between chronic inflammation and tumor development [17]. To better understand the connection between IL-6, signaling proteins and pathways, and proliferative indices, we are currently looking at how IL-6 influences the colonic epithelial cell kinetics in pigs whose gastrointestinal anatomy and physiology, and gut bacterial profile are similar to those of humans compared to any nonprimate animal [60].

Earlier studies in our laboratory and others have shown that anthocyanins and other polyphenols found in purple-fleshed potatoes have anti-inflammatory and anticancer activity [28,44–46,61–65]. Our laboratory has shown that there was a greater reduction in NF-κB activation and in LPS- or TNF-α-induced inflammation in Caco-2 cells with treatment of purple-fleshed potato extracts compared to white potato extracts [66]. Therefore, we chose to investigate the effects of purple-fleshed potatoes on HCD-induced colonic inflammation in a human-relevant animal model. Since potatoes are typically processed before consumption, we chose to investigate if anti-inflammatory properties of the purple-fleshed potatoes are retained after baking. Our findings suggest that purple-fleshed potato supplementation in HCD (10% w/w) suppressed HCD-induced colonic inflammation, shown by the significant down-regulation of IL-6 and proteins identified from proteomics for their involvement in the IL-6 signaling pathway. Interestingly, we saw a greater suppression of HCD-induced IL-6 expression with baked purple-fleshed potatoes than raw. It is possible that, during the processing, the bioactive compounds are more readily available. In a recent study, we showed that baking elevated the total polyphenol and anthocyanin content in potatoes, likely due to their increased extractability [45]. Therefore, it is plausible that these compounds are more readily available for use by the gut bacterial metabolism into short-chain fatty acids [45,67]. However, the raw potato samples were more potent than the baked samples in regulating downstream proteins in the IL-6 signaling cascade. Therefore, further research would need to be completed to validate these findings. This is also in line with our recently published data wherein we showed that baked purple-fleshed potatoes reduced the number of crypts containing cells with nuclear β-catenin (an indicator of colon cancer stem cells) *via* induction of apoptosis and suppressed tumor incidence similar to that of sulindac, a standard therapy drug, in a mouse model of azoxymethane-induced colon tumorigenesis [68].

While therapeutics have been developed for the treatment of colon cancer, including those targeting IL-6, they can have adverse side effects, adding to a patient's stress during disease. Furthermore, chemotherapeutics are expensive, adding a financial burden to families and patients. Plant foods may contain greater levels of polyphenols and are readily available year-round at a local grocery store. These foods, including potatoes, are a very cost-effective method of preventing disease and eliminating the future stress that comes with colon cancer. Our data suggest that purple-fleshed potatoes, even after processing, can reduce HCD-induced inflammation. As proliferating colonocytes are highly susceptible to damage due to oxidative stress/inflammation, the simple, affordable addition of purple-fleshed potatoes to the diet could help prevent and reduce the risk of colon cancer. As dietary changes can readily be made, there is potential for evidence-based plant foods to be used as an affordable dietary therapeutic or preventative care as a natural alternative to drugs.

In conclusion, HCD induced colonic inflammation in the human-relevant porcine model, and IL-6 may be a target gene for inflammation induced by HCD and HCD-linked increased risk for colon cancer. IL-6 and its downstream proteins correlated with proliferative indices in the colon crypt, confirming the link between inflammation and colon

cancer risk [17]. We observed that dietary supplementation of purple-fleshed potatoes, even after processing, prevented HCD-induced inflammation. Ultimately, this work is important as chronic inflammatory environment can lead to a multitude of diseases, including type 2 diabetes and colon cancer. The prevalence of chronic inflammation-promoted diseases are on the rise around the globe; thus, it is critical to develop safe, effective and affordable dietary interventions/strategies to help prevent the onset of diseases.

Author contributions

S.R. conducted the pig study under the supervision of J.V., L.R. and S.W.K. A.S. performed the qPCR (and analyses) and statistical analysis, and correlations. E.E. performed the Ki-67 proliferative index and zone experiments used for correlations. S.R. and A.S. performed the extraction of the samples for proteomics. S.R. worked with J.V. and V.B. for proteomic analysis data collection and analysis. A.S. wrote the manuscript with help from S.R. and J.V. F.S and Q.L conducted the statistical analysis for proteomics data. J.V. was the Principal Investigator of the study. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no competing financial interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jnutbio.2017.01.012.

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