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Dietary whey protein lowers serum C-peptide concentration and duodenal SREBP-1c mRNA abundance, and reduces occurrence of duodenal tumors and colon aberrant crypt foci in azoxymethane-treated male rats☆

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Abstract

We evaluated partially hydrolyzed whey protein (WPH) for inhibitory effects on the development of colon aberrant crypt foci (ACF) and intestinal tumors in azoxymethane (AOM)-treated rats. Pregnant Sprague–Dawley rats and their progeny were fed AIN-93G diets containing casein (CAS, control diet) or WPH as the sole protein source. Colons and small intestines from the male progeny were obtained at 6, 12, 20 and 23 weeks after AOM treatment. At 6 and 23 weeks, post-AOM, WPH-fed rats had fewer ACF than did CAS-fed rats. Intestinal tumors were most frequent at 23 weeks, post-AOM. At this time point, differences in colon tumor incidence with diet were not observed; however, WPH-fed rats had fewer tumors in the small intestine (7.6% vs. 26% incidence, $P = .004$). Partially hydrolized whey protein suppressed circulating C-peptide concentration (a stable indicator of steady-state insulin secretion) at all four time points relative to the corresponding CAS-fed animals. The relative mRNA abundance for the insulin-responsive, transcription factor gene, SREBP-1c, was reduced by WPH in the duodenum but not colon. Results indicate potential physiological linkages of dietary protein type with circulating C-peptide (and by inference insulin), local expression of SREBP-1c gene and propensity for small intestine tumorigenesis.

Keywords: Whey; Insulin; SREBP-1c; Intestine; Colon; Cancer

1. Introduction

Cancers of the large and small intestine are major contributors to worldwide cancer morbidity and mortality [1]. Diets have a profound influence on the incidence of these cancers; those that are low in fiber, calcium and vitamin D but high in fat (the "Western diet") increase colon cancer risk, whereas diets that are low in fat and high in fruits, vegetables, calcium, vitamin D, soy and legumes lower colon cancer risk [2–7]. The physiological and biochemical basis for effects of dietary components on cancers of the small and large intestine remains relatively unclear.

Whey protein, a by-product of the cheese-making process, constitutes ~20% of the total bovine milk protein [8]. Previous studies have found that dietary whey protein isolates can stimulate immune system function, improve muscle strength and body composition, and inhibit cardiovascular disease and osteoporosis [9–11]. Several studies evaluated whey protein in the prevention and/or treatment of cancers (reviewed in Refs. [12–14]). Dietary whey protein concentrate (WPC) inhibited colon tumor incidence, reduced tumor burden and extended life span [relative to casein (CAS) diet] in dimethylhydrazine (DMH)-treated A/J mice [12,15,16]. Diets containing whey protein reduced colon tumor incidence in DMH- or azoxymethane (AOM)-treated Sprague–Dawley rats [5,17,18]. Likewise, diets containing a partial hydrolysate of whey protein (WPH) inhibited colon and mammary carcinogenesis in Sprague–Dawley rats [19,20]. The major protein components of bovine whey are β-lactoglobulin, α-lactalbumin, immuno-

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globulin, albumin, lactoferrin and glycomacropeptide [8]. Purified lactoferrin or its pepsin hydrolysate retarded mouse colon tumor growth and metastasis when provided orally [21–23]. Sphingomyelin and glycosphingolipids, nonproteinaceous components of whey, inhibited the aberrant crypt foci (ACF) occurrence as well as the relative incidence of adenocarcinomas and adenomas in mice [24–26]. Certain whey proteins have cell growth-stimulatory or -inhibitory actions in vitro [27–29]. Mechanistically, however, much remains to be determined of how dietary whey proteins retard cancer initiation and tumor development.

Aberrant crypt foci are putative preneoplastic lesions observed during experimentally induced colon tumorigenesis and are frequently used as an intermediate end point to evaluate nutritional factors and chemopreventative agents in colon carcinogenesis [30–34]. Elevated serum C-peptide (an indicator of insulin secretion) is a prognostic risk factor for colon cancer in humans (reviewed in Ref. [35]). The present study was designed to evaluate the effects of dietary WPH on the temporal appearance of ACF and intestinal tumors as well as the possible relationships of C-peptide and insulin in WPH-mediated inhibition of tumorigenesis. The resultant data identify the effects of AOM and dietary protein type on circulating C-peptide, and by inference, insulin, which may therefore underlie the actions of WPH to suppress duodenal tumorigenesis.

2. Materials and methods

2.1. Animals

Animal protocols were approved by the University of Arkansas for Medical Sciences Institutional Animal Care and Use Committee. Rats were housed in an AAALAC-approved animal facility at the Arkansas Children's Hospital Research Institute. Pregnant Sprague–Dawley dams from Charles River Laboratories (Wilmington, MA) were received at gestation day 4 and immediately placed on diets (CAS or WPH). At postnatal day (PND) 3, each litter was culled to five males and five females. (Females were used in unrelated studies.) Animals were housed in polycarbonate cages in temperature- and humidity-controlled rooms with a daily photoperiod of 12-h light and 12-h dark. Animals were allowed ad libitum access to food and water and were weighed weekly.

2.2. Diets and treatments

Casein (ALACID 741) and WPH (WPH 917) were purchased from New Zealand Milk Products (North America, Santa Rosa, CA). Semipurified, isocaloric, AIN-93G diets [36] containing CAS (20%, w/w) or WPH (20%, w/w) as the sole protein source were prepared as described previously [19,37]. Corn oil replaced soybean oil, and diets were supplemented with cysteine/cystine (CAS), methionine (WPH) or phenylalanine (WPH) for balancing to the AIN-93G formula. Diets were prepared by Harlan-Teklad (Madison, WI) to provide similar amounts of energy, protein, fat, calcium, phosphorus and other trace elements (data not shown). Food intake was measured over a 48-h period using the Oxymax System (Columbus Instruments, Columbus, OH). Casein- and WPH-fed animals were injected subcutaneously with AOM (15 mg/kg body weight, Midwest Research Institute) or saline (CAS only) at ages PND 50 and PND 57, which followed the conventional regimen for AOM treatment of rats [34].

2.3. Tumors/ACF

The experimental design is shown in Fig. 1. Animals were killed at 6, 12, 20 and 23 weeks post-AOM, and serum was prepared and stored at −80°C for later C-peptide assay. The small intestines and colons from all animals were examined for tumors, which were excised, trimmed of mesentery and weighed. To assign regional location to tumors, we divided the small intestine and colon, respectively, into three segments of equal length — designated as proximal, mid and distal. A portion of each dissected tumor was placed in formalin for later examination by a pathologist. All animals at 6, 12 and 20 weeks post-AOM were used for ACF analysis, whereas at 23 weeks post-AOM, 25 random animals per group were used (Fig. 1). Aberrant crypt foci visualization was as previously described [38]. Colons were examined in blinded fashion by one observer; typically, several from each diet group failed to yield usable ACF data due to poor quality of fixation.

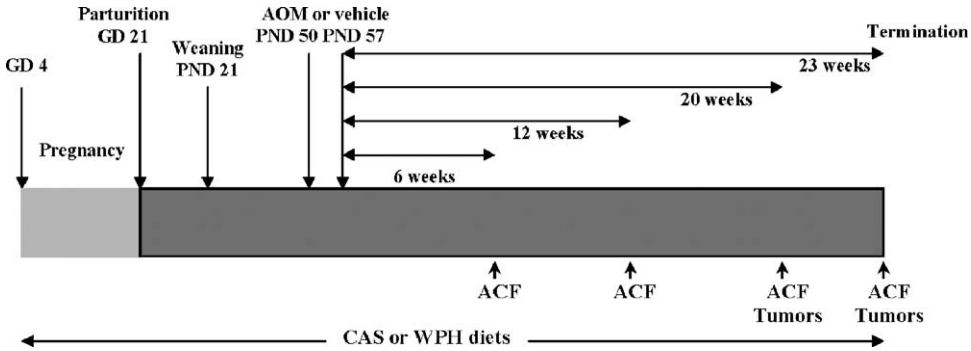

Fig. 1. Experimental design. GD indicates gestation day.

The number of rats that yielded ACF data (considered to be a random effect) is indicated in the legend to Fig. 2.

2.4. C-peptide determination

The rat C-peptide RIA (Linco Research, St. Charles, MO) was used to measure concentrations of C-peptide in sera from the three groups and at the four time points. The assay had a sensitivity of 25 pM and exhibited intra- and interassay variations of less than 6%.

2.5. SREBP-1c mRNA quantification

Ribonucleic acid was isolated from the proximal small intestines and midcolons of nontumored animals at week 23 post-AOM. Non-tumor-bearing animals were used so as to avoid possible confounding effects of tumor and/or nonnormal tissue on mRNA abundance. Ribonucleic acid also was isolated from proximal small intestines at week 6 post-AOM. Complementary DNA was synthesized from 1 μg of proximal small intestine RNA from each animal (n=5–9 animals/group) and from 1 μg of midcolon RNA from each animal (n=5–9 animals/group) using the iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA). The primers for rat SREBP-1c were upstream primer, 5′-CAGAGGGACTACAGGCTGAGAAAG-3′; and downstream primer, 5′-CACGTAGATCTCTGCCAGTGTTG-3′. Cyclophilin mRNA was used to normalize real-time PCR results (primers for rat cyclophilin mRNA: upstream primer, 5′-AAGCATACAGGTCCTGGCATCT-3′; and

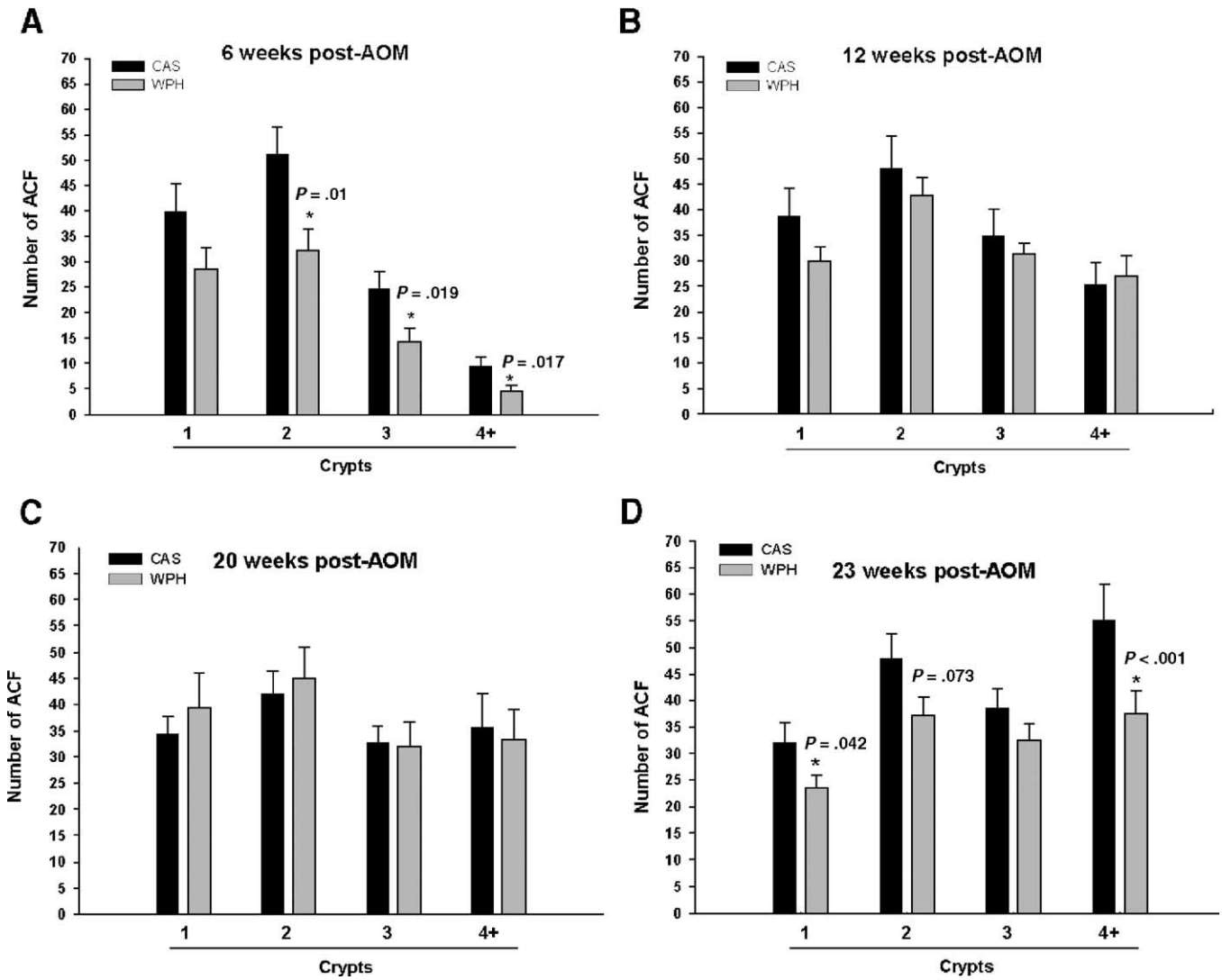

Fig. 2. Effects of CAS and WPH on AOM-induced colon ACF in male Sprague–Dawley rats. Panels A, B, C and D show the ACF occurrence at 6 weeks (n=13 and 13 animals for CAS and WPH, respectively), 12 weeks (n=12 and 15 animals for CAS and WPH, respectively), 20 weeks (n=13 and 11 animals for CAS and WPH, respectively) and 23 weeks (n=19 and 21 animals for CAS and WPH, respectively) after the second of two AOM treatments. Shown are the means±S.E.M. for number of ACF containing one, two, three and four or more (4+) aberrant crypts per ACF. P values indicate significant differences (P<.05) or a tendency (.1>P>.05) for a difference between CAS and WPH for each time point.

downstream primer, 5′-TGCCATCCAGCCACTCAGT-3′). Real-time quantitative PCR was performed as previously described [39].

2.6. Statistical analyses

Statistical analysis was performed using SigmaStat for Windows Version 2.03 (SPSS, Chicago, IL). The effects of dietary protein on number of ACF with one, two, three and four or more crypts, and ACF multiplicity (number of crypts per ACF) were compared using Student's *t* test or the Mann–Whitney rank sum test, the latter when data were not normally distributed. For this purpose, ACF were compared within each time point. Diet effects on tumor incidence were compared using Fisher's Exact Test (week 20) or by χ^2 analysis (week 23). The effects of dietary protein and time after AOM administration (6, 12, 20 or 23 weeks) on ACF frequency and ACF crypt multiplicity were examined by two-way ANOVA followed by the Bonferroni *t* test for all pairwise multiple comparisons. One-way ANOVA or *t* tests were used to compare serum C-peptide concentrations and SREBP-1c mRNA abundance between CAS and WPH groups and between control and AOM-treated CAS-fed groups. Pearson correlation analysis assessed potential associations of C-peptide levels and ACF indices in AOM-treated animals at all four time points. Values are presented as means±S.E.M. Differences between treatment means were considered significant at $P<.05$, whereas $.05<P<.1$ was considered to indicate a tendency for an effect.

3. Results

3.1. Dietary protein type, ACF number and ACF crypt multiplicity

Animals exhibited sigmoidal growth curves. Consistent with a previous study [19], body weights of male rats fed WPH were slightly less than for those fed CAS (4–11% difference in weight depending on the age when examined, $P<.05$). Azoxymethane treatments induced a small but significant ($P<.05$) decrease in growth relative to the CAS group, which was administered saline. Feed intake did not differ between diet groups ($P=.341$).

Tissues were collected at 6, 12, 20 and 23 weeks after AOM or saline treatments. Aberrant crypt foci were not evident in colons from saline-injected animals. At 6 weeks post-AOM, animals fed WPH had fewer ACF containing two crypts ($P=.01$), three crypts ($P=.019$), and four or more crypts ($P=.017$) when compared with corresponding CAS-fed, AOM-treated rats (Fig. 2A). In contrast, no differences in ACF occurrence with dietary protein type were found at 12 and 20 weeks (Fig. 2B, C). Suppressive effects of WPH on ACF frequency were again observed at 23 weeks (Fig. 2D), with the number/animal of ACF with one crypt ($P=.042$) and four or more crypts ($P<.001$) decreased by WPH. Aberrant crypt foci were more prevalent in the mid and distal than the proximal colons at all time points (data not shown).

Two-way ANOVA delineated effects of "diet" and "time" on number of small ACF (one to three aberrant crypts),

Table 1
Effects of diet and time after AOM administration on colon ACF frequency and ACF crypt multiplicity*

	Time post-AOM injection (weeks)				Diet effect
	6	12	20	23	
Number of small ACF					
CAS	115.7 ± 10.0^{a}	121.8 ± 12.1	108.9 ± 11.6	118.4 ± 9.6^{a}	$P<.05$
WPH	74.6 ± 9.5^{b}	103.6 ± 10.8	116.0 ± 12.6	92.8 ± 8.9^{b}	
Time effect	NS				
Number of large ACF					
CAS	$9.4\pm1.9^{a,C}$	22.2 ± 5.5^{BC}	35.7 ± 5.3^{B}	$55.1\pm4.4^{a,A}$	$P<.05$
WPH	$4.4\pm1.2^{b,B}$	26.9 ± 5.0^{A}	33.2 ± 5.8^{A}	$37.5\pm4.1^{b,A}$	
Time effect	$P<.001$				
Total number of ACF					
CAS	125.1 ± 10.3^{a}	147.1 ± 15.8	144.6 ± 15.1	173.4 ± 12.5^{a}	$P<.05$
WPH	$79.0\pm10.1^{b,B}$	130.5 ± 14.1^{A}	149.2 ± 16.5^{A}	$130.2\pm11.6^{b,A}$	
Time effect	$P<.01$				
ACF crypt multiplicity					
CAS	2.05 ± 0.09^{C}	2.47 ± 0.10^{B}	2.64 ± 0.10^{B}	3.00 ± 0.08^{A}	NS
WPH	1.94 ± 0.07^{C}	2.56 ± 0.09^{B}	2.57 ± 0.11^{AB}	2.91 ± 0.08^{A}	
Time effect	$P<.001$				

^{a,b} Indicate significant differences ($P<.05$) within a column (diet effect).
^{A,B,C} Indicate significant differences within a row (effect of time, post-AOM).
Two-way ANOVA (Bonferroni *t* test for all pairwise multiple comparisons).

* Data are representative of the entire colon: small ACF are those with one, two or three crypts; large ACF are those with four or more crypts; total ACF=no. of small ACF+no. of large ACF; crypts/ACF=total crypts/total no. of ACF/animal; overall diet effect — the ANOVA *P* value is shown; overall time effect — the ANOVA *P* value is shown. No ACF were observed in any of the saline-treated controls.

Table 2
Azoxymethane-induced intestinal tumor incidence and location

Rats per group	Control[a]	20 weeks post-AOM		23 weeks post-AOM	
	CAS	CAS	WPH	CAS	WPH
	16	15	15	73	79
% tumor-bearing animals					
PC	0	6.7	0	11.0	6.3[b,c]
MC	0	33.3[d]	13.3	21.9	19.0
DC	0	0	0	20.5	21.5
Entire colon	0	40	13.3	39.7	38.0
Proximal small intestine	0	20	6.7	26.0[e]	7.6
Middle small intestine	0	6.7	0	0	0
Distal small intestine	0	0	0	0	0
Entire small intestine	0	26.7	6.7	26.0[e]	7.6
Overall	0	60[f]	20	52.1	41.8

Proximal colon indicates PC; middle colon, MC; distal colon, DC.
Week 20, Fisher's Exact Test; week 23, χ^2 test.
[a] Five control rats (vehicle injected) killed at 20 weeks; 11 control rats killed at 23 weeks.
[b] WPH (week 23, MC) vs. WPH (week 23, PC), $P=.031$.
[c] WPH (week 23, DC) vs. WPH (week 23, PC), $P=.011$.
[d] CAS (week 20, MC) vs. CAS (week 20, DC), $P=.042$.
[e] CAS (week 23) vs. WPH (week 23), $P=.004$.
[f] CAS (week 20) vs. WPH (week 20), $P=.06$.

number of large ACF (four or more aberrant crypts), total ACF number (sum of small and large ACF) and ACF crypt multiplicity (aberrant crypt number/ACF number). Dietary WPH inhibited overall occurrence of small ACF ($P<.05$), large ACF ($P<.05$) and total number of ACF ($P<.05$); however, ACF crypt multiplicity was unaffected by dietary protein type (Table 1). Interestingly, the time post-AOM had no effect ($P>.1$) on the number of small ACF for either diet group. For both diets, however, the number of large ACF ($P<.001$) as well as ACF crypt multiplicity ($P<.001$) were increased over time. There was no statistical interaction of diet and time for ACF number or ACF crypt multiplicity. Taken together, the data indicate that WPH suppresses occurrence of small as well as large ACF without any effect on ACF crypt multiplicity, and moreover, point to the time-dependent emergence of large ACF from an apparently stable pool of small ACF.

3.2. Dietary protein type and intestinal tumorigenesis

Whey protein hydrolysate-fed rats exhibited a trend ($P=.06$) for diminished total tumor incidence in combined small intestine and colon at 20 weeks (Table 2). A significant decrease (70%; $P=.004$) in total tumor incidence in the small intestine was observed in WPH-fed rats at week 23. Regional trends in tumor location were apparent. At week 20, tumors predominated in the middle one-third of the colon, and the proximal one-third of the small intestine (duodenum) (Table 2). At week 23, more tumors were observed in the middle and distal than proximal regions of the colon; all small intestine tumors were in the duodenum (Table 2). No tumors were observed in the saline-treated, control animals.

Tumors were classified as adenomatous polyps (AP), APs with carcinoma in situ, invasive adenocarcinomas, invasive mucinous carcinomas or metastatic adenocarcinomas with signet ring features. Signet ring carcinomas were found only at week 23 and localized to the proximal colon (one tumor in CAS and WPH groups, respectively) and proximal small intestine (one tumor in the CAS group). Invasive mucinous carcinomas were observed only at week 23. This latter tumor was most frequent in the proximal small intestines of CAS animals (six tumors), whereas fewer such tumors were found in proximal small intestine of the WPH group (one tumor) and in proximal colon (CAS, two tumors; WPH, one tumor). Whey protein hydrolysate-fed animals exhibited a trend ($P=.091$) for diminished tumor multiplicity in colon plus small intestine at 23 weeks after AOM treatment; however, no effects of diet on tumor weight were observed.

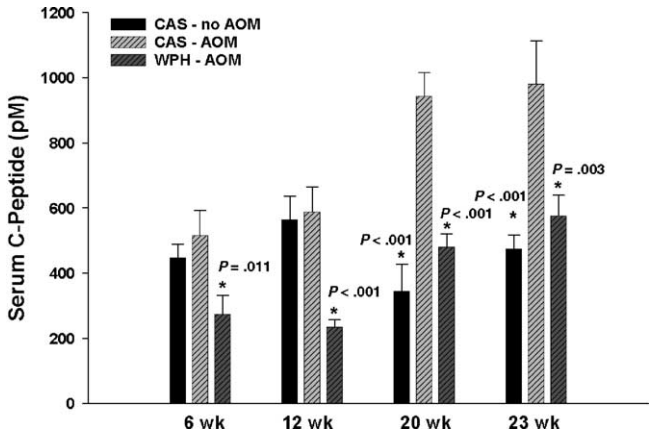

Fig. 3. Effects of AOM and WPH on serum C-peptide concentration (means±S.E.M.); P values indicate significant differences between WPH-AOM-treated ($n=10$) or CAS-vehicle-treated ($n=5$) animals compared with CAS-AOM-treated ($n=10$) animals within each time point.

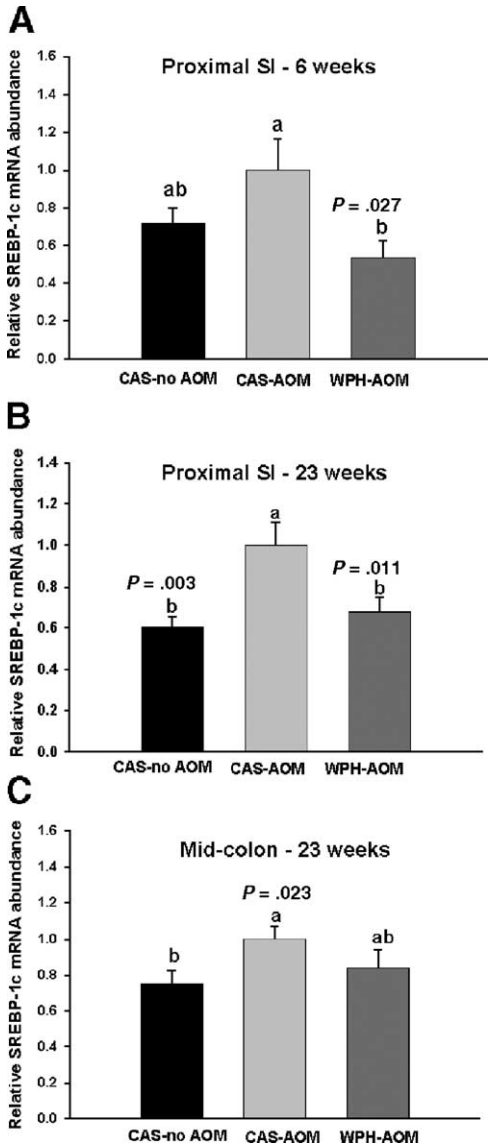

Fig. 4. SREBP-1c mRNA abundance in proximal small intestine (SI) (A, B) and midcolon (C) of nontumored animals at 6 weeks (A) and 23 weeks (B, C) post-AOM. Real-time reverse transcription PCR quantification of SREBP-1c mRNA was performed and the data normalized to cyclophilin mRNA. The mean value for the CAS-AOM group was set at 100%. *P* values indicate significant differences between the WPH-AOM and CAS-AOM groups as well as the CAS-vehicle-treated and CAS-AOM groups.

3.3. Serum C-peptide

The concentration of C-peptide in sera was determined in relation to ACF/tumor incidence. Azoxymethane treatments resulted in elevated serum C-peptide concentrations in CAS-fed animals at 20 and 23 weeks ($P<.001$ and $P<.001$, respectively) when compared with the corresponding CAS-fed, saline-treated controls (Fig. 3). However, these differences were not observed for 6 and 12 weeks post-AOM. Whey protein hydrolysate diet fed to AOM-treated rats decreased serum C-peptide concentration at 6 ($P=.011$), 12 ($P<.001$), 20 ($P<.001$) and 23 ($P=.003$) weeks, relative to the corresponding CAS-fed, AOM-treated rats (Fig. 3). A tendency for a positive correlation ($r=.43$, $P=.09$) of circulating C-peptide concentration and total ACF number at 6 weeks post-AOM was noted. However, no correlations of ACF number with C-peptide were found at 12, 20 or 23 weeks, post-AOM (data not shown).

3.4. Intestinal SREBP-1c mRNA

Typically, SREBP-1c mRNA abundance is induced in response to insulin. As shown in Fig. 4, SREBP-1c mRNA abundance in proximal small intestine at 6 and 23 weeks was significantly reduced in WPH-fed, AOM-treated animals ($P<.03$) when compared with the CAS-fed, AOM-treated animals. Examination of the midcolon at 23 weeks demonstrated elevated SREBP-1c mRNA abundance with AOM treatment ($P=.023$), but no differences between CAS- and WPH-fed, AOM treated animals. At 23 weeks post-AOM, the mRNA results for small intestine mirrored the observed differences in serum C-peptide concentration (Figs. 3 and 4). Intestinal tissues corresponding to 12 and 20 weeks were not analyzed for SREBP-1c mRNA.

4. Discussion

We report the novel effects of WPH, in the diet, on the timing of appearance of colon ACF and on the incidence of duodenal tumors in AOM-treated, male Sprague–Dawley rats. We also demonstrate that WPH elicits reductions in serum C-peptide levels and insulin target gene expression in duodenum, which are temporally associated with reduced tumor occurrence in this tissue region.

We observed a significant reduction with WPH in the frequency of small and large ACF at 6 and 23 weeks after AOM. The absence of a dietary protein effect on ACF prevalence at 12 and 20 weeks may reflect catch-up in the number of initiated ACF in the WPH group, whereas the differences observed at 23 weeks may reflect the emergence of new and/or more complex ACF that are inhibited by WPH (or conversely stimulated by CAS) in AOM-treated rats. In this regard, others have reported the appearance of a second emergent cohort of ACF in AOM-treated F344 rat colon at ~30 weeks after AOM treatments [40]. In the present study, ACF crypt multiplicity increased in time-dependent fashion as expected [41], but this was unaffected by the type of dietary protein type. This result indicates the inhibition by WPH of ACF initiation rather than ACF progression, as increases in ACF crypt multiplicity are generally considered a measure of the latter.

In male Wistar rats, ACF were accurate predictors of latter-occurring adenocarcinomas and adenomas in distal colon, but not of carcinomas of signet ring cell type, the latter tumor type occurring in the proximal colon [40,42]. We observed greater numbers of tumors in the middle and distal rather than the proximal regions of the colon, and this mimicked the regional differences in ACF occurrence. Moreover, we observed two signet ring cell tumors localized to the proximal colon, in agreement with

the above-described localization of this tumor type. Mucinous carcinomas are more prevalent in proximal than distal regions of the colon [43]. We similarly observed these tumors only in the proximal region of the colon.

In humans, small intestine adenocarcinomas and mucinous carcinomas predominate in the duodenum or the proximal aspect of the jejunum; the relative risk for developing such cancers is influenced by dietary factors [44–46]. We observed a substantial number of invasive adenocarcinomas and invasive mucinous carcinomas in the proximal small intestine of the CAS-fed animals. This localization is in good agreement with the published work for rat models [47] and buttresses use of the AOM-treated rat for modeling human small intestinal carcinogenesis and its dietary correlates.

The physiological mechanisms by which whey proteins inhibit intestinal tumorigenesis remain to be established. When compared with CAS, whey proteins and peptides have faster transit times within the gastrointestinal tract and increased digestion rates [48–51]. Whey peptides may differentially affect small intestine uptake and transfer of macromolecules and nutrients as well as functioning of the enteroendocrine system [39,52,53]. In a seminal study, Bounous et al. [15] reported reduced tumor multiplicity and tumor size in A/J mice fed WPC when compared with CAS-based diet. Comparison of amino acid profiles for the proteins in this previous study indicated a major difference only in cysteine (0.3 vs. 2.3 g/100g protein, CAS and WPC, respectively). Indeed, whey proteins are rich sources of cysteine and glutamylcysteine [17], and this was postulated to confer the anticancer actions of whey by favoring synthesis of intracellular glutathione [12]. However, in the present study, CAS and WPH diets had nearly identical content of cysteine/cystine and methionine as confirmed by quantitative analysis of the final diets (data not shown), and therefore, this cannot be the basis for the observed differences in the biological effects of CAS and WPH observed here.

In a previous report, feeding of whey protein led to lower "fasting" insulin levels when compared with dietary red meat [48]. Ingestion of WPH is known to result in rapid postprandial increases in venous plasma total amino acids and, as a consequence, rapid but transient increases in circulating insulin, whereas dietary CAS results in a slower but more sustained elevation in these same responses over time [50,51,54]. Our data indicate a greater overall insulin secretion with CAS than WPH diets when fed to AOM-treated rats, and this was associated with the observed greater weights of the CAS-fed animals. In this regard, exogenous insulin is known to stimulate growth of AOM-treated rats [55]. The hypothesis that dietary WPH elicits overall lower insulin (as reflected by the observed decrement in C-peptide, a stable indicator of insulin secretion) and which may underlie antitumorigenesis is supported by published data. Long-term administration of insulin to AOM-treated rats resulted in increased colon tumor incidence (79% vs. 50% for controls) and increased tumor multiplicity (2.0 vs. 0.73 for controls) [55]. Daily administration of exogenous insulin to AOM-treated F344 rats increased ACF crypt multiplicity [56]. In humans, elevated plasma C-peptide concentrations are correlated with increased risk for CRC [57,58]. Intriguingly, C-peptide may manifest bioactivities, including binding to specific cell surface receptors and activating downstream signaling pathways [59]. Whether these activities explain the association of C-peptide with increased colon cancer risk remains to be established.

The influences of WPH and AOM on circulating C-peptide and intestinal SREBP-1c mRNA levels and the known association of elevated fatty acid synthesis with tumorigenesis [60,61] implicate SREBP-1c in the anticancer mechanism of WPH. Induction of fatty acid synthase (FAS) expression with consequently enhanced synthesis of fatty acids is required for neoplastic transformation and tumor progression [61]. Mitogen-activated protein kinases and phosphoinositide-3-kinase are the main signaling cascades responsive to insulin [62], and SREBP-1c is an important transcriptional intermediary for insulin stimulation of FAS gene expression. Based on the present data, we suggest that a decrease in overall insulin output elicited by dietary WPH serves to dampen SREBP-1c expression in the small intestine, thereby reducing cell proliferation and/or survival of initiated tumor cells. Additional studies are required to confirm the proposed functional linkages of WPH-based diets, insulin, SREBP-1c and tumorigenesis and to probe the underlying molecular mechanism. In summary, long-term consumption of WPH is protective against the development of chemically induced duodenal cancers of the rat. On the basis of the present results, we propose that this effect is due to the reduced circulating levels of C-peptide (and insulin), the known risk factors for cancers of the colon and, as suggested here, the small intestine.

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