Clinical Nutrition 31 (2012) 60–64

Contents lists available at SciVerse ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Colonocyte telomere shortening is greater with dietary red meat than white meat and is attenuated by resistant starch

Nathan J. O'Callaghan[a,*], Shusuke Toden[b], Anthony R. Bird[b], David L. Topping[b], Michael Fenech[a], Michael A. Conlon[b]

[a] Nutritional genomics and DNA Damage Research Group, CSIRO Food and Nutritional Sciences, Kintore Avenue, PO Box 10041, Adelaide, SA 5000, Australia
[b] CSIRO Food Futures National Research Flagship, CSIRO Food and Nutritional Sciences, Kintore Avenue, Adelaide, Australia

ARTICLE INFO

Article history:
Received 24 March 2011
Accepted 1 September 2011

Keywords:
Resistant starch
Red meat
White meat
Telomere
Colonocytes

SUMMARY

Background & aims: Population studies indicate that greater red meat consumption increases colorectal cancer risk while dietary fibre is protective. Previous work in rats showed that diets high in protein, including red meat, increase colonocyte DNA strand breaks and that this effect is attenuated by resistant starches (RS). Telomeres are long hexamer repeats that protect against spontaneous DNA damage which would lead to chromosomal instability. Telomere shortening is associated with greater risk of colorectal cancer. The current study aimed to determine the effects of cooked red and white meat intake on colonocyte telomere length in rats and whether dietary RS modified their effects. Methods: After four weeks of feeding cooked beef or chicken at 15, 25 and 35% of diet with or without RS, colonocyte telomere length was measured. Results: Telomere length decreased in proportion to red meat content of the diet. A similar trend was observed in the white meat group. Colonocyte telomere shortening due to increased dietary meat was attenuated by the inclusion of RS. Conclusion: These data support previous findings of increased colonocyte DNA damage with greater red and white meat intake and also the protective effect of dietary fibre.

1. Introduction

Diet is an important factor in the aetiology of important non-infectious diseases, including colorectal cancer (CRC).[1–5] Prospective cohort studies have shown that greater dietary intakes of red and processed meats, but not white (chicken and fish) meats, are associated with increased CRC risk while the consumption of fibre is protective; this effect is particularly strong at low dietary fibre intakes.[6–10] Genetic damage can initiate cancer and we have shown in rats, that diets high in a number of protein sources induce colonocyte DNA strand breaks as measured by the comet assay.[10] However, we noted differences between protein sources and damage was much greater with red than white meat, consistent with the relative risk identified in population studies.[1–5,10–14] In all cases, inclusion of resistant starches (RS) in the experimental diets opposed the damage induced by high dietary protein. RS is emerging as an important contributor to total dietary fibre and its intake is high in some population groups at low risk of CRC who consume traditional diets.[8,9] The protective effect of RS appears to be mediated, at least partly, through the short chain fatty acids (SCFA) produced by its fermentation by the large bowel microflora.[15] These acids promote optimal large bowel function and one SCFA in particular (butyrate) is thought to be the most effective in this regard, acting to promote a normal cell phenotype through a number of complimentary mechanisms.

Telomeres are long hexamer (TTAGGG) repeats that protect the genome against chromosomal instability and cellular senescence.[16–19] Telomere degradation has been shown to increase instability via end-fusion and the generation of breakage-fusion-bridge cycles within chromosomes. These changes in genome stability are important initiating events in cancer and ageing related disorders.[20–22] Telomere shortening within the colon and rectum is a hallmark of colorectal carcinogenesis.[23,24] There is increasing evidence that diet related factors lead to telomere shortening. Most recently data from the Nurses Health Study indicate that shorter leukocyte telomere length was associated with increased processed meat intake, while dietary fibre intake was positively associated with greater telomere length.[25] Despite this growing associative evidence on dietary factors that may affect telomere length, the

Abbreviations: RS, resistant starch; HAMS, high amylose maize starch; SCFA, short chain fatty acids.

* Corresponding author. Tel.: +61 8 8303 8867; fax: +61 8 8303 8899.
E-mail addresses: nathan.ocallaghan@csiro.au, oca018@csiro.au (N.J. O'Callaghan).

0261-5614/$ – see front matter © 2011 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.
doi:10.1016/j.clnu.2011.09.003

specific impact that increased red meat intake and increased RS has on telomere length in colorectal mucosa is unknown. Given the crucial role these DNA structures have in CRC risk, it was important to determine whether telomeres are influenced by dietary red and white meat and whether these effects are modulated by RS within the colon.

2. Materials and methods

2.1. Animals and diets

Adult, male Sprague-Dawley rats ($n = 96$) weighing approximately 200 g were obtained from the Animal Resource Centre, Murdoch University, Perth, Western Australia. Rats were housed in wire-bottomed cages in a room of controlled heating and lighting (23 °C with a 12-h light/dark cycle) and had free access to food and water. Animals were randomly allocated to 12 groups ($n = 8$ per group) and fed one of 12 diets for 4 weeks as described previously.[10]

The diets, based on the AIN-93 diet,[26] contained 15, 25 or 35% red meat (beef round rump boneless steak trimmed of fat) with or without 20% high amylose maize starch (HAMS) (*Hi-maize*™, National Starch and Chemical Company NSW, Australia). Meat, purchased from Central Market Meat (Adelaide, SA), were cooked on a hotplate with a temperature of 150 °C until lightly browned. The meat was then dried at 45 °C for 48 h and milled. The beef contained 73.4% protein and 18.3% fat. Diets without HAMS contained highly digestible starch (cornstarch, National Starch and Chemical Company) instead. The ileal digestibility of HAMS and the highly digestible starch were previously measured in rats and shown to be 58% (i.e. 42% RS) and 99%, respectively.[27] All diets contained 5% wheat bran as the fibre source. At the conclusion of the study the rats were anaesthetised with 4% halothane/oxygen and gut tissues, hepatic portal and aortic bloods were collected for analyses. All procedures involving animals were approved by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Human Nutrition Animal Ethics Committee and the University of Adelaide Animal Ethics Committee.

2.2. Tissue collection, DNA isolation and telomere length measurements

Colon cells were isolated via tissue scraping of the dissected tissue. Cells were placed into PBS and placed at −20 °C until required. DNA was isolated using a QIAGEN DNeasy Kit with minor modifications, as described previously.[28,29] Absolute telomere length (aTL) was measured by determining the number of TTAGGG hexamer repeats using quantitative real-time PCR (qPCR) as described.[29] aTL values are reported as kb/diploid genome and were calculated using a synthesised TTAGGG 84mer oligonucleotide to generate a standard curve; the number of diploid genome copies per reaction was determined using the single copy gene *36B4*.

2.3. Malondialdehyde (MDA), caecal fermentation product and Comet measurements

Concentrations of MDA in colon, total and major individual short chain fatty acids (SCFA) acetate, propionate and butyrate, and phenol and cresol concentrations in large bowel digesta in these animals were measured and previously reported.[10,30] Double and single strand breaks (DSB and SSB respectively) in colonocytes of these animals were determined by Comet assay and previously reported.[10]

Table 1
Effects of resistant starch with increasing meat intake on telomere length of colonocytes, DNA damage and caecal fermentation products.

Dietary Meat (%)	Red Meat						White Meat					
	without HAMS			with HAMS			without HAMS			with HAMS		
	15%	25%	35%	15%	25%	35%	15%	25%	35%	15%	25%	35%
aTL (kb/genome)	1894^{a}	1317^{ab}	673^{b}	1873^{a}	1960^{a}	1686^{a}	1951^{a}	1787^{a}	1388^{a}	2514^{a}	2119^{a}	1863^{a}
COMET												
Single strand breaks	743^{abc}	1103^{d}	1461^{e}	592^{ac}	572^{ac}	608^{ac}	655^{ac}	844^{abd}	987^{bd}	549^{c}	635^{ac}	722^{abc}
Double strand breaks	379^{ac}	417^{ab}	476^{b}	296^{de}	286^{e}	287^{e}	314^{cde}	339^{cde}	362^{acd}	302^{de}	286^{e}	300^{e}
Colonic Malondialdehyde (nmol/mg)	0.29^{ab}	0.34^{a}	0.42^{c}	0.27^{ab}	0.30^{ab}	0.30^{ab}	0.29^{ab}	0.25^{b}	0.34^{a}	0.27^{ab}	0.28^{ab}	0.29^{ab}
Caecal SCFA Pools (μmol)												
Acetate	64^{a}	105^{b}	73^{a}	255^{c}	216^{c}	308^{c}	61^{a}	68^{a}	70^{a}	238^{c}	217^{c}	226^{c}
Propionate	18^{a}	25^{b}	19^{ab}	95^{c}	84^{cd}	97^{c}	18^{a}	20^{ab}	19^{a}	54^{e}	71^{de}	63^{e}
Butyrate	18^{a}	30^{b}	21^{ac}	53^{d}	78^{e}	43^{d}	17^{a}	18^{a}	22^{c}	52^{d}	56^{de}	45^{d}
Total caecal SCFA	105^{a}	170^{b}	121^{ac}	412^{d}	389^{de}	457^{d}	102^{a}	112^{ac}	119^{c}	355^{e}	357^{e}	346^{e}
Caecal phenol (ug/g)	6.7^{a}	4.8^{ab}	4.1^{b}	0.4^{cd}	0.4^{cd}	0.5^{c}	2.8^{b}	5.8^{ab}	3.8^{b}	0.3^{d}	0.3^{d}	0.5^{c}
Caecal *p*-cresol (ug/g)	5.5^{ab}	17.9^{c}	20.7^{c}	3.3^{ad}	4.6^{ab}	5.9^{b}	3.5^{ad}	5.1^{ab}	22.7^{c}	3.7^{ad}	1.1^{e}	2.9^{de}

$n = 8$ per group; data are presented as bar graphs representing mean aTL ± SEM. Columns not sharing a letter in common are significantly different (Tukey's Post-Hoc, $P < 0.05$). Data extracted from previous studies: comet, SCFA, phenol and p-cresol[10] and MDA.[30]