

THE INTERACTION BETWEEN DIETARY PROTEINS AND RESISTANT STARCH ON LARGE BOWEL HEALTH

**A thesis submitted to the University of Adelaide for the degree of Doctor of
Philosophy**

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July 2007

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DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or tertiary institution, and to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give my consent to this thesis, when deposited in the University library, being available for photocopy or loan.

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Shusuke Toden

ACKNOWLEDGEMENTS

I would like to start by thanking my primary supervisor Dr. Michael Conlon for guidance, encouragement and support throughout my post-graduate study. You have given me a great degree of freedom and thank you for believing in me. I would like to thank Dr. David Topping for supervising me and also developing my skill as a scientist. Furthermore, more thanks goes to Assoc. Prof. Pat Buckley, Assoc. Prof. Mike Nordstrom, Dr. Tony Bird and Prof. Tim Miles for wise advices and support.

I would also like to thank Phil Thomas for not only being a great mate but also being my role model and helping me progress as a scientist. A special thanks goes to Damien Belobrajdic, Tanya Lewanowitsch, Nathan O'Callaghan, Grant Brinkworth, Jenny McInerney, Balazs Bajka, Catherine Seccafien and Roger King for supports.

My work could not have been completed without the help of Ben Scherer, Debbie Davies, Caroline Cooke, Corinna Bennett, David Courage and Jacqui Rickard for their assistance in animal handling and biochemical assays.

This project would not have been possible without the financial assistance of CSIRO Food Futures National Research Flagship and CSIRO Division of Human Nutrition. The University of Adelaide travelling grants and Discipline of Physiology scholarships must also be acknowledged for their additional research funding.

Finally I would like to thank my family and friends for their support, in particular my grand mother for encouragements and motivations.

STATEMENT OF AUTHORSHIP

Publication 1:

RESISTANT STARCH PREVENTS COLONIC DNA DAMAGE INDUCED BY HIGH DIETARY COOKED RED MEAT OR CASEIN IN RATS

Toden, S., Bird, A.R., Topping, D.L. & Conlon, M.A.

Cancer Biology and Therapy (2006) Mar;5(3):267-72

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Publication 2:

DIFFERENTIAL EFFECTS OF DIETARY WHEY, CASEIN AND SOYA ON COLONIC DNA DAMAGE AND LARGE BOWEL SCFA IN RATS FED DIETS LOW AND HIGH IN RESISTANT STARCH

Toden, S., Bird, A.R., Topping, D.L. & Conlon, M.A.

British Journal of Nutrition (2007) 97(3):535-543

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Publication 4:

HIGH RED MEAT DIETS INDUCED GREATER NUMBERS OF COLONIC DNA DOUBLE-STRAND BREAKS THAN WHITE MEAT IN RATS: ATTENUATION BY HIGH AMYLOSE MAIZE STARCH

Toden, S., Bird, A.R., Topping, D.L. & Conlon, M.A.

Carcinogenesis (2007) In Press

TODEN, S. (Candidate)

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ABSTRACT

A review of the literature revealed that diet plays an important role in serious human non-infectious large bowel diseases including cancer and inflammatory bowel diseases. Dietary protein (especially as red and processed meats) has been implicated as a positive risk factor for colorectal cancer while starch which is not digested in the small intestine (resistant starch, RS) appears to be protective. The series of experiments described in this thesis were aimed to determine the effects of dietary proteins and RS on indices of colon health in an animal model, the laboratory rat. Genetic damage is a prerequisite for carcinogenesis and this was assessed by a specific assay (the comet assay) which gives a measure of DNA strand breaks. Loss of mucus barrier function is thought to contribute to inflammatory bowel disease by permitting bacterial translocation and this was measured optically using a microscope micrometer. Other biomarkers were measured as described below. There were four major experiments.

1. Effects of dietary red meat and casein on colonic DNA damage and interaction with resistant starch

Previous studies had shown that higher dietary protein (as casein) induced genetic damage in rat colonocytes and that RS (fed as a high amylose maize starch) was protective. This study was aimed at establishing whether a high protein diet fed as cooked red meat had similar effects and whether RS was protective. Rats were fed diets containing either 15 % or 25% casein or 25% barbecued lean red beef, each with or without 48% high amylose maize starch (as a source of RS) for 4 weeks. As expected, high dietary casein caused a 2-fold increase in colonic single-strand DNA breaks compared with a low casein diet and

reduced the thickness of the colonic mucus layer by 41%. High levels of cooked meat caused 26% more DNA damage than the high casein diet but reduced mucus thickness to a similar degree as casein. Addition of RS to the diet abolished the increase in DNA damage and the loss of colonic mucus thickness induced by either high protein diet. It is thought that RS promotes large bowel health through the SCFA produced by the large bowel bacteria. One acid in particular (butyrate) has been associated particularly with promotion of normal large bowel function and protection against disease. In keeping with this hypothesis, caecal and faecal short chain fatty acid pools (including those of butyrate) were increased by inclusion of RS in the diet. DNA damage is an early step in the initiation of cancer and these findings agree with the population data which suggest that total dietary protein and red meat promote risk of colorectal cancer. However, inclusion of resistant starch in the diet could significantly reduce that risk.

2. Differential effects of dietary whey, soy and casein on colonic DNA damage and interaction with resistant starch

The preceding experiments showed that high levels of animal-derived proteins increased colonocyte genetic damage and loss of the mucus barrier in rats. This second experiment was designed to determine whether diets high in different types of dairy protein (casein or whey) or a plant protein isolate (soy) had similar adverse effects on colonic DNA and mucus barrier function and whether inclusion of RS in the diet was protective. Adult male Sprague Dawley rats were fed a diet containing 15 % or 25 % casein, whey or soy protein, each with or without 48 % high amylose maize starch for 4 weeks. In confirmation of the earlier studies, higher levels of dietary casein increased colonocyte DNA damage significantly. However, whey did not increase genetic damage. Colonic DNA damage

was highest for soy when fed at both 15% and 25% protein in the absence of RS. Inclusion of RS in the diet attenuated colonocyte DNA damage due to higher dietary protein in all three groups. The colonic mucus barrier was thinner in rats fed higher dietary protein but the effect was reversed by feeding RS. Caecal total SCFA and butyrate pools were low in rats fed the digestible starch and were higher in rats fed RS. However, there was no relationship between caecal or faecal SCFA and genetic damage or mucus thickness. Caecal and colonic tissue weight and colon length were higher in rats fed RS, consistent with greater SCFA supply. These data confirm that higher dietary protein of animal (casein) or plant (soy) origin increases genetic damage and loss of the mucus barrier indicating that this is an effect of protein and not its source. These findings accord with the epidemiological data which link dietary protein to greater risk of colorectal cancer and inflammatory bowel disease. However, the data show also that dietary proteins differ in their specific actions on genetic damage and mucus thickness. Further, the data from the feeding of whey suggest that not all proteins are equivalent in their capacity to provoke adverse changes in colonic integrity. While the data show that RS raised large bowel and faecal SCFA, they indicate their levels were not related directly to these biomarkers.

3. Dose response effects of resistant starch on protein induced colonic DNA damage

The accumulated data linking greater protein intakes to adverse changes in the colon were obtained at dietary levels which were not unreasonable in terms of animal or human consumption. However, the dietary level of RS which were fed were relatively high (48% by weight) so this study was conducted to determine its effectiveness at lower levels of

dietary inclusion. It was also important to ascertain whether there was a dose-response relationship between RS intake and the observed effects. One of the mechanisms proposed for the induction of colorectal cancer by high dietary protein intakes is oxidative damage to DNA. In this experiment, this was done by assaying with endonuclease III. Adult male rats were fed a diet containing 25% casein with 0%, 10%, 20%, 30% or 40% high amylose maize starch for 4 weeks. As in the preceding studies comet tail moment was greatest and the mucus barrier thinnest in rats fed 0% RS. DNA damage was reduced and the mucus barrier thickened in a logarithmic dose-dependent manner by RS. There was no significant difference between dietary groups associated with oxidative DNA damage as measured by endonuclease III. Caecal and faecal short chain fatty acid (SCFA) pools rose with the increased level of dietary RS. DNA damage of colonocytes correlated negatively with caecal SCFA but the strongest correlation was with caecal butyrate, which is consistent with the proposed role of this SCFA in promoting a normal cell phenotype. The data show that RS prevents protein induced colonic DNA damage in a dose-dependent manner. Inclusion of 10% high amylose maize starch was found to be sufficient to oppose colonocyte DNA damage, and to increase caecal and faecal SCFA pools. Intakes of this order are not unreasonable in terms of human consumption of RS.

4. Dose response effects of red and white meat on colonic DNA damage and interaction with resistant starch

The accumulated evidence from large prospective human studies links diet to colorectal cancer risk strongly. The evidence from the animal studies described in this thesis that dietary protein induces colonocyte genetic damage supports a role for high protein intakes in increasing risk. Recently, several large epidemiological studies and a meta-analysis of

prospective studies have found that consumption of dietary red or processed meats, but not white (poultry) meat, is associated with increased risk of colorectal cancer. This is consistent with the data from the preceding studies that specific proteins affected colonic integrity differentially. A large prospective European study (European Prospective Investigation into Cancer and Nutrition) has reported that dietary fibre was protective. The findings reported in this thesis that RS opposes the effects of high dietary protein accord with that conclusion. This study aimed to compare the effects of cooked red (beef) or white (chicken) meat on DNA damage and mucus barrier thickness in rats. The study was designed to determine whether the relationship between the intakes of these meats was dose-dependent. Double-strand DNA breaks are thought to relate more closely to carcinogenesis than single-strand breaks so both were measured. Adult male Sprague-Dawley rats were fed a diet containing 15%, 25% or 35% cooked beef or cooked chicken each with or without 20% high amylose maize starch for four weeks. Both red and white meat increased colonic DNA damage dose-dependently. However, both single and double strand breaks were significantly greater when the rats were fed the red meat diets compared to those fed the white meat. Colonocyte DNA damage was reduced by the consumption of RS while large bowel SCFA were increased. The findings of this study are consistent with the epidemiological data which show that red meat consumption is associated with greater risk of colorectal cancer but that white meat is not.

Summary

The data reported in this thesis support the findings of prospective population studies that high dietary protein, red meat in particular, appears to be harmful to the health of the large bowel. However, the data demonstrate also that different protein types have differential

effects on the integrity of the colonocyte DNA. Furthermore, the addition of RS to the diet protects against protein-induced colonic DNA damage and maintenance of the colonic mucus barrier, apparently through increased SCFA production by colonic fermentation. The results of these experiments indicate a strong potential for RS to be effective in maintenance of large bowel integrity in the face of high dietary protein.

PUBLICATIONS ARISING FROM THESIS

Toden, S., Bird, A.R., Topping, D.L. & Conlon, M.A. High Red Meat Diets Induce Greater Numbers of Colonic DNA Double-Strand Breaks than White Meat In Rats: Attenuation by High Amylose Maize Starch. *Carcinogenesis* 2007 *In Press*

Toden, S., Bird, A.R., Topping, D.L. & Conlon, M.A. Dose-dependent reduction of dietary protein-induced colonocyte DNA damage by resistant starch in rats is more highly correlated with levels of caecal butyrate than other short chain fatty acids. *Cancer Biology and Therapy* Feb; 26(2):253-258, 2007

Toden, S., Bird, A.R., Topping, D.L. & Conlon, M.A. Differential Effects of Dietary Whey, Casein and Soy on Colonic DNA Damage in Rats. *British Journal of Nutrition* 97(3):535-543, 2007

Toden, S., Bird, A.R., Topping, D.L. & Conlon, M.A. Resistant starch prevents colonic DNA damage induced by high dietary cooked red meat or casein in rats. *Cancer Biology and Therapy* Mar;5(3):267-72, 2006

Toden, S., Bird, A. R., Topping, D. L., and Conlon, M. A. Differential effects of dietary whey and casein on colonic DNA damage. *Aust. J. Dairy Technol.* 60, 146-148 2005

PRESENTATIONS

Conference Abstracts

Conlon, M.A., **Toden, S.**, Bird, A.R., Topping, D.L. Resistant starch and colonic mucosal integrity. Asia Pacific Journal of Clinical Nutrition **15 Suppl:** S45, 2007

Toden, S., Bird, A. R., Topping, D. L., and Conlon, M. A. Resistant starch protects against colonic DNA damage induced by dietary whey, soy and casein in rats Dietary Fibre 2006 Multifunctional Complex of Components 160-161, 2006

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ABBREVIATIONS

ACF	aberrant crypt foci
APC	adenomatous polyposis coli
AIN	American Institute of Nutrition
ANOVA	analysis of variance
BaP	benzo(a)pyrene
cm	centi-meter
CRC	colorectal cancer
°C	degrees, Celsius
DNA	deoxyribonucleic acid
DMEM	Dulbecco's Modified Eagle's Medium
DMH	dimethylhydrazine
DTT	dithiothreitol
DSB	double-strand break
FAP	familiar polyposis
g	grams
GSH	glutathione
GST	glutathione S transferase
h	hours
HBSS	Hanks' balanced salt solution
HCA	Heterocyclic amines
HAMS	High amylose maize starch,
HDAC	histone deacetylase

IBD	Inflammatory bowel disease
M	molar
µg	micrograms
µl	milligrams
mm	millimetres
min	minutes
NOC	N-nitrosocompounds
NSP	non-starch polysaccharides
PBS	phosphate buffered saline
PAH	Poly aromatic hydrocarbons
pH	potential of hydrogen
PhIP	2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
RS	Resistant starch,
SCFA	short chain fatty acid
SEM	standard error of the mean
SSB	single-strand break
TCF	transcription factor
TBS	tris buffered saline
wk	weeks