British Journal of Nutrition (2003), **89**, 283–284
DOI: 10.1079/BJN2002744

Nutrition Discussion Forum

Diets enriched with cereal brans or inulin modulate protein kinase C activity and isozyme expression in rat colonic mucosa – Comments by Pool-Zobel & Cherbut

Pajari *et al.* (2000) compared the effect of selected dietary fibres, including inulin, wheat bran, oat bran and rye bran, on the colon and colonic mucosa of rats. A group receiving a fibre-free diet was included as a negative control. The key endpoints were determinations of protein kinase C (PKC) activity and protein levels by immunoblotting. Specifically, the authors measured membrane PKC δ and cytosolic PKC α, ζ and λ in tissue of the proximal colonic mucosa, and membrane PKC $\beta 2$ and cytosolic PKC $\beta 2$, δ, ζ and λ in the distal colonic mucosa. PKC-membrane activities were also determined in the distal and proximal colonic mucosa.

There appears to be some confusion in the presentation of the results. Quite logically, data were compared for rats fed fibre-containing diets and those fed a fibre-free diet. However, in some cases data were also compared for rats fed diets containing different fibres, which led to puzzling conclusions. For the results comparing rats fed a fibre-free diet (control) and those fed a fibre-containing diet, the statistically significant results with inulin were: (1) higher weight of caecum plus contents; (2) higher weight of caecal mucosa; (3) higher stool weight; (4) higher membrane PKC δ level in the proximal colon; (5) higher cytosolic PKC ζ and λ; (6) higher membrane:cytosol PKC activity in the proximal colon (but the methodology is not described); (7) higher cytosolic PKC β2 protein level in the distal colon.

Findings 1 to 3 are the usual effects observed for fermentable carbohydrates in general (Jacobs & Lupton, 1984; Key *et al.* 1996), including inulin (Remesy *et al.* 1993; Fontaine *et al.* 1996), and are also considered as beneficial, health-promoting mechanisms of various fibre sources (Scheppach *et al.* 2001). However, the PKC findings have not previously been reported in healthy inulin-fed rats, and the authors' conclusions in this respect seem unlikely: 'However, ingestion of the inulin-enriched diet resulted in an increased PKC activity and PKC β2 level in the distal colonic mucosa, indicating that this highly-fermentable fructose polymer may enhance colon carcinogenesis.' As noted below, these conclusions are based on results that are statistically non-significant (in part) and not always consistent with data in the Results section of the paper. Finally, the conclusions are speculative, as they imply that the measured parameters (PKC in healthy rat mucosa) are predictive of carcinogenesis.

The following comments suggest that the authors' conclusions are misleading:

(1) The statement that 'ingestion of the inulin-enriched diet resulted in an increased PKC activity and PKC β2 level in the distal colonic mucosa' appears to be incorrect, as PKC activity was not significantly modulated in the distal colonic mucosa. The reported increase of activity was found in the proximal mucosa, but not in the distal mucosa. Moreover, there is no indication of how PKC activity was measured.

(2) The statement that inulin 'enhances colon carcinogenesis' because of 'increased PKC activity and PKC β2 level' is highly speculative, as it has not been established that PKC-modulation directly reflects carcinogenic processes and can serve as a predictive parameter (Hofmann, 2001). Protein kinase constitutes a family of at least twelve different isozymes, each believed to play distinct regulatory roles for cell proliferation, differentiation and apoptosis. For instance, PKC β2 is probably involved in cell growth, and PKC δ activation is likely to induce apoptosis. For these reasons, PKC modulation leads to many contradictory results. Moreover, even if PKC activity could be considered as a marker of cell proliferation, it is not certain that increases in cell proliferation induced by fibre consumption would be predictive of increased tumorigenesis (Whiteley & Klurfeld, 2000).

(3) The conclusions are not justified in terms of the 'state of the art' at the time the paper was written. In this respect, the authors quoted several sources in support of PKC involvement in carcinogenesis, such as: 'Several lines of evidence suggest that PKC β2 mediates colonic cell proliferation and that elevated PKC β2 expression and activity enhance colon carcinogenesis. In carcinogen-injected animals both PKC β2 expression and membrane–particulate association were increased in tumours relative to the uninvolved surrounding mucosa (Wali *et al.* 1995), suggesting that membrane association of PKC β2 may be related to the growth advantage of tumour cells' (Discussion, paragraph 2). However, all data in the authors' study were obtained in animals that had not been injected with carcinogens, and the parameters were measured in healthy, non-tumour-bearing tissue.

(4) Other contradictions relate to statements made in the last paragraph of the Discussion: 'In conclusion, the present study demonstrated that diets enriched with different fibre sources could have very different effects on colonic PKC activity and isozyme expression. Specifically feeding of the wheat-bran-enriched diet resulted in a low distal PKC activity and isozyme expression of PKC β2, a PKC isozyme related to colonic cell proliferation and increased susceptibility for colon carcinogenesis. The favourable effects of wheat bran on PKC activity and isozyme expression may explain in part the protective effect of wheat bran against tumour development in a number of experimental colon cancer studies. However, ingestion

of the inulin-enriched diet resulted in an increased PKC activity and PKC β2 level in the distal colonic mucosa.'

The PKC activity in the distal colon of wheat-bran-fed rats was 2107 (SD 518) pmol/min per mg protein, a finding not statistically different (i.e. not decreased) in comparison with that for non-fibre control rats (2424 (SD 914) pmol/min per mg protein). The corresponding value for inulin-fed rats was 2773 (SD 778) pmol/min per mg protein, which is also not statistically different from the control (i.e. not increased).

Membrane PKC β2 levels in the distal mucosa were 0·09 (SD 0·03) relative intensity units for the wheat diet and 0·12 (SD 0·04) for the non-fibre control. These findings were not statistically different (and thus not decreased in the wheat-bran-fed group). The corresponding value for inulin-fed rats was 0·17 (SD 0·05) relative intensity units, which is also not statistically different from the control (and thus not increased).

The authors support their conclusion that inulin is a 'carcinogenesis enhancer' by citing data from their previous study: '…fructose polymer may enhance colon carcinogenesis. This possibility is supported by the result of our latest study which demonstrated that inulin at a level of 25 g/kg diet promoted intestinal tumour development in Min mice (Mutanen *et al.* 2000)'. However, this possibility seems questionable as well. The 'cancer-enhancing' properties of inulin are based mainly on higher yields of small-intestinal tumours than for rye bran-fed animals. In fact, there was no significant difference between inulin-fed animals and those from the non-fibre-fed control group. Even the reported significant differences between rye- and inulin-fed animals are not scientifically acceptable, as the diets of cereal-fed *APC*^{Min} mice had a different composition from those of inulin-fed *APC*^{Min} mice (which contained at least 10 % higher levels of fat and protein).

Finally, another finding of importance for the conclusions reached by Pajari *et al.* (2000) is that the PKC data reported for *APC*^{Min} mice showed no associations at all with tumour sites, so that this parameter was non-predictive of carcinogenesis in APC mice that actually develop tumours. These observations are in striking contrast with the way PKC data were interpreted in healthy rats.

It is of major importance to study the possibly detrimental effects of food ingredients. In particular, potentially chronic health effects should be carefully investigated and evaluated with the best techniques available. However, the conclusions reached by Pajari *et al.* (2000) seem suspect. The notion of the potential 'cancer-enhancing effects of inulin' was based on non-significant results in comparison with controls or on supportive evidence from another equally questionable study (Mutanen *et al.* 2000). Moreover, the major endpoint used to define inulin as a carcinogenesis enhancer was the detection of a single protein group from a key signal transduction pathway (PKC), as measured in healthy colonic mucosa of rats that had not been injected with a carcinogen. As this pathway is not only important for tumorigenic processes, but also for physiological functions, it is not surprising that PKC were detected in epithelial tissue, which is continuously undergoing renewal, especially in animals fed a highly fermentable fibre such as inulin. Moreover, PKC are not established predictors of carcinogenesis. Even in their own studies in *APC*^{Min} mice, the same group of authors found no associations between cancer and PKC expression, which makes the conclusion that PKC are predictors of carcinogenesis even more unconvincing. Due caution is needed before implying that a food ingredient has potential '*carcinogenesis enhancing activity*' especially one that has been shown to have several beneficial effects in the colon of experimental animals and human subjects. Only scientifically valid results can provide an acceptable basis for such suppositions.

Beatrice Pool-Zobel

Institute of Nutrition, Friedrich Schiller University
Dornburger Str. 25, Jena
D-07743 Germany

Christine Cherbut

Human Nutrition Research Centre, INRA
BP 71627
44316 Nantes cedex 03
France

References

Fontaine N, Meslin JC, Lory S & Andrieux C (1996) Intestinal mucin distribution in the germ-free rat and in the heteroxenic rat harbouring a human bacterial flora: effect of inulin in the diet. *British Journal of Nutrition* **75**, 881–892.

Hofmann J (2001) Modulation of protein kinase C in antitumor treatment. *Review of Physiology, Biochemistry and Pharmacology* **142**, 1–96.

Jacobs LR & Lupton JR (1984) Effect of dietary fibers on rat large bowel mucosal growth and cell proliferation. *American Journal of Physiology* **246**, G378–G385.

Key FB, McClean D & Mathers JC (1996) Tissue hypertrophy and epithelial proliferation rate in the gut of rats fed on bread and haricot beans (*Phasoleus vulgaris*). *British Journal of Nutrition* **76**, 273–286.

Mutanen M, Pajari AM & Oikarinen SI (2000) Beef induces and rye bran prevents the formation of intestinal polyps in *Apc*^{Min} mice: relation to β-catenin and PKC isozymes. *Carcinogenesis* **21**, 1167–1173.

Pajari A-M, Oikarinen S, Gråsten S & Mutanen M (2000) Diets enriched with cereal brans or inulin modulate protein kinase C activity and isozyme expression in rat colonic mucosa. *British Journal of Nutrition* **84**, 635–643.

Remesy C, Levrat MA, Gamet L & Demigné C (1993) Cecal fermentations in rats fed oligosaccharides (inulin) are modulated by dietary calcium level. *American Journal of Physiology* **264**, G855–G862.

Scheppach W, Luehrs H & Menzel T (2001) Beneficial health effects of low-digestible carbohydrate consumption. *British Journal of Nutrition* **85**, S23–S30.

Wali RK, Frawley BP Jr, Hartmann S, Roy HK, Khare S, Scaglione-Sewell BA, Earnest DL, Sitrin MD, Brasitus TA & Bissonnette M (1995) Mechanism of action of chemoprotective ursodeoxycholate in the azoxymethane model of rat colonic carcinogenesis: potential roles of protein kinase C-alpha, -beta II, and -zeta. *Cancer Research* **55**, 5257–5264.

Whiteley LO & Klurfeld DM (2000) Are dietary fiber-induced alterations in colonic epithelial cell proliferation predictive of fiber's effect on colon cancer? *Nutrition and Cancer* **36**, 131–149.