British Journal of Nutrition (2003), **89**, 285–286
DOI: 10.1079/BJN2002745

Nutrition Discussion Forum

Diets enriched with cereal brans or inulin modulate protein kinase C activity and isozyme expression in rat colonic mucosa–Reply by Pajari & Mutanen

With interest, we noticed that our results published nearly two years ago (Pajari *et al.* 2000) still give rise to extensive attention as it appears in the lengthy letter of Drs Pool-Zobel and Cherbut. It is also interesting that Drs Pool-Zobel and Cherbut focus entirely on inulin when disagreeing with us in three main aspects: (1) how to use statistics and present results; (2) what is the role of protein kinases C (PKC) in colon carcinogenesis and their use as predictive markers of tumour formation; (3) how to interpret and discuss PKC β2 data.

(1) *Statistics*. The main reason for disagreement on data interpretation appears to be the view of Drs Pool-Zobel and Cherbut on how to use statistics. They would only take into consideration differences between fibre-containing diets and a fibre-free diet. This is of course one of the possible approaches and is actually the way it used to be when one used Student's *t* test in multiple comparisons without the help of computers. However, the main focus in our paper was not on inulin, as Drs Pool-Zobel and Cherbut seem to presume, but to compare different fibre sources in their effects on PKC activity and expression in the rat colon. The statistical approach was chosen accordingly, i.e. ANOVA and Tukey's *post hoc* test, which is actually very strict concerning statistical significance (more strict than Student's *t* test) and allows multiple comparisons. In fact, it provides protection for significant differences by chance when multiple comparisons are made. We feel that this approach is more appropriate than mere pairwise comparisons and does more justice for the data since the different fibre sources clearly have opposite effects on PKC activity and expression. Traditional use of the *t* test to compare the treatment groups only against the fibre-free group would partly miss this important point, although we would have obtained more statistically significant results; for example, $P=0{\cdot}033$ for the difference in membrane PKC β2 in the distal colon between the inulin and fibre-free groups. In our paper we have discussed the differences seen against the fibre-free diet and the wheat-bran diet. Wheat bran served as a positive control for its known cancer-preventive effects in the rat. Therefore, the statistical significance between wheat-bran and inulin groups could be very meaningful. In real life, it really can make a difference whether one chose to consume inulin instead of wheat bran for years or even a lifetime. The approach used is clearly stated in the Introduction and Statistical methods in our paper.

(2) *Role of PKC in carcinogenesis*. Drs Pool-Zobel and Cherbut seem to be confused about the importance of different PKC in the carcinogenic process because PKC have diverse physiological functions. Furthermore, they mention 'PKC modulation' as a non-predictive parameter of carcinogenesis, leaving it unclear whether they mean PKC activity or isozyme expression. To our understanding, PKC are not just 'a single protein group' but key players in maintenance of balance between cell proliferation, differentiation and apoptosis in the normal intestinal epithelium. Abundant data also support involvement of PKC in neoplastic transformation and tumour development (see Black, 2001). Depending on their physiological functions, each isozyme has a distinct role in colon carcinogenesis, some of them promoting and others inhibiting the tumorigenic process. For example, PKC ε triggers proliferative signals and is a known oncogene, whereas PKC δ is apoptotic and known to reverse neoplastic process (Weller *et al.* 1999). Furthermore, evidence also exists that, for example, faecal PKC β2 and PKC ζ could serve as biomarkers for colon cancer (Davidson *et al.* 1998). By analysing each isozyme separately, as we have done in our paper, makes interpretation of results unambiguous. Regarding PKC activity, caution is needed because no such substrate exists that would be specific for just one isozyme. Therefore we have focused on the isozyme results in our paper and used the activity results to support the isozyme results. The statement of Drs Pool-Zobel and Cherbut that there is no indication of how PKC activity was measured is not correct either since we have cited one of our earlier publications, which includes detailed description of PKC activity analysis (Pajari *et al.* 1998).

(3) *PKC β2*. When stating that our conclusions on PKC β2 were not justified in terms of the state of the art at the time the paper was written, Drs Pool-Zobel and Cherbut have ignored the most important reference in our discussion on PKC β2. The study of Murray *et al.* (1999) clearly shows that mere overexpression of PKC β2, without carcinogen-treatment, leads to hyperproliferation of colonic epithelium. Later on, when administered to AOM, PKC β2-overexpressing mice also had increased susceptibility to aberrant crypt foci (ACF) formation in the colon. It is a well-known fact that pure overexpression of a protein is one of the mechanisms leading to transformation of cells and thus to neoplastic growth of tissue. Therefore, if steady-state expression and/or activity of PKC β2 are increased in healthy colon mucosa by diet, it really can make these animals prone to tumorigenesis. Thus, our interpretation of the PKC β2 data was not based on comparison between healthy *v.* carcinogen-treated tissue though the results obtained in carcinogen-treated rats gave overall support to our conclusions.

The latest research further confirms our conclusions by showing that an increase in PKC β2 expression occurs early in colon carcinogenesis and directly promotes development of ACF and colon tumours (Gökmen-Polar *et al.*

2001). The promotive effect of PKC β2 on tumorigenesis is likely to be mediated by its role in the APC–β-catenin pathway, where it regulates cellular β-catenin levels through inhibiting GSK3 activity (Cook *et al.* 1996). This is really significant taken that the *APC* tumour-suppressor gene is mutated in 80 % of sporadic colon cancer cases and that the main tumour-suppressor activity of APC protein is its ability to down regulate cellular β-catenin (Kinzler & Vogelstein, 1996). In our Min mice study, feeding inulin resulted in a statistically significant increase in cytosolic β-catenin when compared with the fibre-free group (Mutanen *et al.* 2000). Drs Pool-Zobel and Cherbut have totally ignored this important result on which we partly based our conclusions on inulin. Although not quite significant because of the strict statistical approach, mean adenoma number per mouse in the small intestine was 35 in the non-fibre group and 49 in the inulin group ($P=0{\cdot}07$) and tumour incidence in the colon was 71 % in the non-fibre *v*. 100 % in the inulin group. Together with the β-catenin result we think this means promotion rather than inhibition. Not to detect any dietary differences in PKC β2 in Min mice is no wonder since the neoplastic process at the end of the feeding period could easily have been beyond the state in which diet can modulate PKC expression. Furthermore, we unfortunately did not have the opportunity to analyse PKC β2 separately in adenoma and normal-appearing mucosa tissues, which might have masked tissue-specific changes by diet in PKC β2 expression.

Anne-Maria Pajari and Marja Mutanen
Department of Applied Chemistry and Microbiology
PO Box 27 00014
University of Helsinki
Finland

References

Black J (2001) Protein kinase C isozymes in colon carcinogenesis: guilt by omission. *Gastroenterology* **120**, 1868–1872.

Cook D, Fry M, Hughes K, Sumathipala J, Woodgett J & Dale T (1996) Wingless inactivates glycogen synthase kinase-3 via an intracellular signaling pathway which involves a protein kinase C. *EMBO Journal* **15**, 4526–4536.

Davidson LA, Aymond CM, Jiang Y-H, Turner ND, Lupton JR & Chapkin RS (1998) Non-invasive detection of fecal protein kinase C βII and ζ messenger RNA: putative biomarkers for colon cancer. *Carcinogenesis* **19**, 253–257.

Gökmen-Polar Y, Murray NR, Velasco MA, Gatalica Z & Fields AP (2001) Elevated protein kinase C βII is an early promotive event in colon carcinogenesis. *Cancer Research* **61**, 1375–1381.

Kinzler KW & Vogelstein B (1996) Lessons from hereditary colon cancer. *Cell* **87**, 159–170.

Murray NR, Davidson LA, Chapkin RS, Gustafson WC, Schattenberg DG & Fields AP (1999) Overexpression of protein kinase C βII induces colonic hyperproliferation and increased sensitivity to colon carcinogenesis. *Journal of Cell Biology* **145**, 699–711.

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

Pajari A-M, Häkkänen P, Duan R-D & Mutanen M (1998) Role of red meat and arachidonic acid in protein kinase C activation in rat colonic mucosa. *Nutrition and Cancer* **32**, 86–94.

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.

Weller SG, Klein IK, Penington RC & Karnes WE Jr (1999) Distinct protein kinase C isozymes signal mitogenesis and apoptosis in human colon cancer cells. *Gastroenterology* **117**, 848–857.