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Selenoprotein gene expression in an intestinal cell line during selenium depletion: a macroarray approach indicates effects on SelW and glutathione peroxidase 1. By V. PAGMANTIDIS¹, S. VILLETTE¹, G. BERMANO², J. BROOM², J.R. ARTHUR³ and J.E. HESKETH¹, ¹*School of Cell and Molecular Biosciences, University of Newcastle, Newcastle-upon-Tyne NE1 7RU*, ²*University of Aberdeen, Medical School, Aberdeen, AB24 3FX* and ³*The Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB*

The micronutrient selenium (Se) is incorporated into a range of selenoproteins involved in numerous biochemical processes within the body. There is some debate that the decrease in selenium intake of the UK population over recent years may have implications for health (Rayman, 2002). It is therefore of interest to define how the expression of the various selenoproteins alters when Se supply is suboptimal. There is evidence that when Se supply is limiting there are cellular mechanisms which prioritise selenoprotein synthesis (Hesketh & Villette, 2002). Se deficiency leads to decreases in both protein and mRNA levels of some selenoproteins; this is thought to be due to decreased mRNA translation and a subsequent increased mRNA degradation (Bermano *et al.* 1996a). Genomic approaches present the opportunity to examine effects of Se supply on expression of a range of selenoproteins rather than individual genes. In this study, we have used a custom-made macroarray to study expression of a range of selenoprotein genes.

Caco-2 cells were grown in Dulbecco's Modified Eagle Medium supplemented with 10% fetal calf serum until they reached confluence. They were then maintained in serum-free medium with either insulin (5 µg/ml) and transferrin (5 µg/ml) (Se-deficient cells) or insulin, transferrin and selenium as sodium selenite (7 ng/ml) (Se-replete cells). Under such conditions both Caco-2 and rat hepatoma H4 cells provide a model of Se depletion (Bermano *et al.* 1996b; Pagmantidis *et al.* 2002). Total RNA was extracted, and 3.5 µg of it was used for synthesis of ³²P-labelled cDNA probe by reverse transcription. RNA isolated from Se-supplemented and Se-depleted cells was hybridised overnight in parallel to two identical custom-made 'selenoprotein' AtlasTM arrays. The array contained cDNA probes corresponding to the majority of known human selenoproteins, proteins implicated in selenium metabolism and a limited number of genes whose products are associated with arachidonic acid metabolism and immune response. After washing, specifically bound probe was detected using a phosphorimager and the two array scans were analysed and compared using the AtlasImage v2.7 software. In order to allow for any differences in labelling efficiency between RNA samples, normalisation was performed by taking into account changes in intensity of signals corresponding to the 'housekeeping genes'. Differential gene expression was assessed by calculation of the ratio of signal intensity for the Se-deficient cells to signal intensity for the Se-adequate cells, both corrected for background, and normalised as described above. A change of equal to or greater than two-fold was considered significant.

The results confirmed those found previously by Northern hybridisation (Pagmantidis *et al.* 2002), namely that the mRNA levels for GPX1 and GPX4 decreased under Se depletion, whereas for GPX2 there was no statistical significant change. In addition, expression of SelW was found to decrease in Se depletion. In conclusion, the major changes in selenoprotein gene expression at the mRNA level seen in Caco-2 cells during Se depletion were the marked decrease in abundances of SelW and glutathione peroxidase 1 mRNAs. It is likely that this reflects the instability of these mRNAs under conditions of low Se supply. The relationship of these changes to potential roles of selenium in the prevention of colon cancer is under investigation.

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Rayman MP (2002) *Proceedings of the Nutrition Society* **61**, 203–215.