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Non-alcoholic compounds in beer have protective effects in Caco-2 cells by increasing the expression of genes in the *Keap1–Nrf2* pathway

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Non-alcoholic compounds in beer, such as polyphenols, have been proposed to have beneficial effects on human health and moderate consumption is linked to a reduced risk of adverse chronic disease outcomes[1]. These protective effects are thought to occur via an upregulation of genes in cytoprotective pathways. We hypothesised that beer extract would upregulate genes in antioxidant pathways, contributing to a reduction of total oxidative stress. To address this we analysed expression of genes in human intestinal Caco-2 cells following exposure to dealcoholised beer extract.

Caco-2 cells were seeded and prepared as previously described[2]. Cells were exposed to Dulbecco's modified Eagle medium containing degassed, dealcoholised freeze-dried beer extract for 24 hours. Control cells were exposed to medium alone. RNA was extracted from homogenised Caco-2 cells using TRIzol, and analysed via RNA probe target hybridisation using Affymetrix microarrays. Differences between beer extract-treated and control Caco-2 cells were indicated when there was a fold change in expression of >50 % in either direction, with a significance cut off of $p < 0{\cdot}05$. Data was analysed using Metacore GeneGo software which produced a list of significantly altered biological processes and pathways. To confirm the microarray results, RT-PCR was performed on those genes which were indicated as being significantly altered within the *Keap1–Nrf2* pathway.

GeneGo analysis showed significant ($p < 0{\cdot}001$) alterations in the following oxidative stress pathways: **'**Role of Sirtuin1 and PGC1α in activation of antioxidant defence system', 'Activation of NOX1/2, DUOX1/2 NADPH oxidases' and 'Angiotensin II-induced production of ROS'. The expression of twelve genes within the Sirtuin1 & PGC1α antioxidant defence system were altered to a significant level ($p < 0{\cdot}05$). To confirm the microarray results, RT-PCR was performed on seven genes from the *Keap1–Nrf2* pathway. Data from microarray and RT-PCR experiments were converted to fold changes of increased gene expression as to be directly comparable. The increases in fold expression from RT-PCR were: HMOX1 (+23·9), GCLreg (+7·5), GCLcat (+4·4), SQSTM1 (+3·3), KEAP1 (+2·4), SLC7A11 (+2·2), NQO1 (+2·1). All RT-PCR data for control versus beer extract were significant $p < 0{\cdot}01$. The changes in NRF2 did not achieve significance. The microarray and RT-PCR results showed a positive correlation relationship ($p < 0{\cdot}0001$) of changes in expression in the seven *Keap1–Nrf2* pathwaygenes.

These data suggest that the non-alcoholic compounds in beer increase expression of multiple genes in the *Keap1–Nrf2* oxidative stress pathway. Although changes in Nrf2 itself were not significant, the pathway relies on an increase in 'free' rather than total Nrf2[3]. Further research is required understand the molecular mechanisms involved in modulation of the *Keap1–Nrf2* pathway before any practical or clinical application can be considered.

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