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Impact of increasing fruit and vegetable intake for 12 weeks on cellular immune responsiveness in healthy subjects with low habitual intakes: A pilot investigation

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Low fruit and vegetable (FV) intake is associated with increased risk of chronic diseases such as cardiovascular disease and cancer[(1)]. The health benefits of FV are partly attributed to their phytochemical content, including carotenoids[(2)], reported *in vitro* as modulators of immune function and inflammation. We aim to test whether increasing FV intake in free-living subjects affects T-lymphocyte responsiveness to antigen challenge.

We recruited a sub-sample (*n* 9/group) of healthy volunteers from an existing randomised-controlled trial where subjects with a habitually low FV intake ($\leqslant 3$ portions/d) received an additional 7 FV portions/d (FV group) or consumed their usual diet (control group) for 12 weeks. Subjects (*n* 7 male, *n* 11 female) were 49·7 (SE 1·36) years old with BMI 26·6 (SE 0·79) kgm^{-2}. Mononuclear cells from peripheral blood were collected before and after intervention, and the T-lymphocytes were stimulated in culture with a panel of memory-specific (tuberculin, *C. albicans* cell wall extract, timothy grass extract) or naïve (keyhole limpet haemocyanin) antigens selected to elicit different response types. Cell proliferation was measured by thymidine incorporation assay. Cytokine responses characteristic of major T helper effector (Th) and regulatory (Treg) lymphocyte subsets (Th1, IFN-γ; Th2, IL-4; Th17, IL-17A; Treg, IL-10 and TGF-β_1) were quantified by ELISA. Data were expressed as the ratio of responses in antigen-stimulated versus unstimulated wells. Treg cells were also enumerated in whole blood by flow cytometry. Plasma carotenoids were measured by HPLC.

Compared with the control group, supplementation with FV may help to rebalance the effector and regulatory arms of adaptive immunity, with reductions close to significance for pro-inflammatory responses, lower proliferative responses, and, importantly, higher Treg numbers. For example, pro-inflammatory IFN-γ and IL-17A responses to tuberculin decreased by 42 % ($P = 0{\cdot}054$) and 68 % ($P = 0{\cdot}059$) respectively in the FV group compared with the control group, alongside an expected decrease in proliferative responses and an expected increase in Treg frequency. Principal component analysis of pro-inflammatory and proliferative responses of memory T-lymphocytes shows that the first component scores, which reflect a weighted average of these responses, were significantly different between the control and FV groups ($P = 0{\cdot}019$). Plasma carotenoids, lutein/zeaxanthin and retinol, increased by 52 % ($P = 0{\cdot}005$) and 13 % ($P = 0{\cdot}037$) respectively in the FV group compared with the control group. No significant changes in other cytokines or naïve T-lymphocyte responsiveness were observed and inter-individual variation in responses was high.

Taken together, our results provide an indication for greater regulation of T-lymphocyte inflammatory responsiveness after increased FV consumption by individuals with low intakes, but the impacts are limited. Our *ex vivo* assays provide a more sensitive approach than static systemic measurements, and the effects of supplementation would be identified more clearly by a study with larger sample size.

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