Proceedings of the Nutrition Society (2010), **69 (OCE3)**, E237 doi:10.1017/S0029665110000261

3rd Immunonutrition Workshop, 21–24 October 2009, Girona, Spain

Toll-like receptor 2 activation is essential to induce a T_H1 shift in human PBMCs by plant sterols and plant stanols

F. Brüll, R. P. Mensink and J. Plat
Department of Human Biology, Maastricht University, Maastricht, The Netherlands

Plant sterols and stanols are well known for lowering LDL cholesterol. There are suggestions that these compounds also affect the immune system, as evidenced by an increased cytokine production by Thelper-1 (T_H1) cells in humans after sitosterol–glucoside consumption or in mice after plant sterol consumption. Whether plant stanols also have these effects is not known. In addition, it is not known if the effects of plant sterols and stanols on cholesterol metabolism *in vivo* interact with these effects.

The objectives of our studies were to evaluate at constant cholesterol concentrations (1) whether sitosterol and sitostanol induce a T_H1 shift in peripheral blood mononuclear cells (PBMC) *in vitro* and (2) to explore the underlying mechanism.

For this, freshly isolated human PBMCs or a cultured U937 monocyte cell line were stimulated to proliferation with PHA or PMA, respectively, and incubated with physiological concentrations of sitosterol or sitostanol for 52 h. Conditions with similar amounts of cholesterol were used as a control condition. Changes in cytokine production were measured using ELISAs, antibody arrays and flow cytometry. Toll-like receptor (TLR)2 and TLR4 agonists and blockers were used to determine the role of TLRs. Finally, PBMCs isolated from TLR2 −/− mice were used to confirm findings in the blocking experiments.

Sitosterol as well as sitostanol increased the production of the T_H1 cytokines IFNγ and IL-2 as compared to control and cholesterol conditions in PHA stimulated PBMCs, while no changes in the T_H2 cytokines IL-10 or IL-4 were seen. The increase of IFNγ production was dose dependent. There were no detectable changes in the production of the U937 monocyte-derived cytokines IL-12 and IL-18. Blocking TLR2 blunted the increase in IFNγ production by sitosterol and sitostanol back to control levels, whereas TLR4 blocking did not have this effect. The role of TLR2 was confirmed by the absence of effects of plant sterols on cytokine production in PBMCs from TLR2 (−/−) as compared to wild-type mice.

In conclusion, sitosterol and sitostanol induce a T_H1 shift in PBMCs *in vitro*. This effect was independent of cholesterol concentrations. Activation of TLR2 seems an essential step in the underlying pathway. Functional effects of this T_H1 shift in humans await evaluation in future studies. If our findings can be extrapolated to the human *in vivo* situation, stimulating the T_H1 response by plant sterols/stanols might be useful in conditions characterised by T_H2 overactivation, which opens a complete new application field for these accepted functional food ingredients.