Research stay at the University of Southampton, United Kingdom: Genetic, inflammation and nutrition - The interaction of n-3 polyunsaturated fatty acids on atherosclerosis

Project: 333

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In September 2004 I was given the opportunity to participate in a study group at the Institute of Human Nutrition of Southampton University. My research stay was financially supported by a grant from the SFEFS Swiss Foundation for Nutrition Research. The research group, led by Prof. Robert F. Grimble and Prof. Philip Calder, studies the interaction between nutrition and inflammation as well as the impact of cytokine biology on metabolic effects depending on the genetic background. The objective is to provide an insight into how nutrients could be used to change the course of infection and inflammatory disease in beneficial ways.

In a prospective study (SoFiA - Study of Fish oil in Atherosclerosis) on 97 male patients with peripheral arterial disease (PAD) we investigated the inflammatory potential of monocytes in correlation with the cytokine genotype and the intake of n-3 PUFA (polyunsaturated fatty acids). The clinical endpoints were ankle brachial pressure index (ABPI) and the walking distance measured on a treadmill. The approach goes back to the findings of the same research group, that demonstrated for the first time, that fish oil reduced activity of macrophages in atheromatous plaques, thereby contributing to plaque stability (Thies et al. Lancet 2003, 361: 477-485).

We collected biometrical and clinical data, blood and urinary samples both before and 12 weeks after a daily intake of 6g fish oil (1.02g EPA and 0.69g DHA). After cell separation and staining blood for surface markers, macrophage cultures were set up and partially stimulated with LPS (lipopolysaccharide). Cells, cytokines, surface markers and adhesion molecules were categorized and counted by flow cytometry (FACS Bead Array). Lipid diagnostic on plasma and monocytes was carried out by gas chromatography. PCR technique was used to classify the genotype focussing on cytokine relevant alleles, especially IL 1β -511, IL 10 –1082, IL 6 – 174, LTα /TNFβ +252, TNFα -308 und TNFα -376. Simultaneously to the quantitative measurements adhesion and migratory behaviour of neutrophils, monocytes and lymphocytes were studied at the Department of Physiology at Birmingham University by video-microscopy in capillary co-cultures of endothelial cells and macrophages, which were separated before (Luu et al. Atherosclerosis 2006 Sep 16. Comparison of the pro-inflammatory potential of monocytes from healthy adults and those with peripheral arterial disease using an in vitro culture model).

At the annual congress of the European Society of Clinical Nutrition and Metabolism (ESPEN) in Brussels in autumn 2005 we presented the first results of our work. Our abstract

- Fish oil reduces pain and improves walking distance in peripheral vascular disease: an effect modulated by the TNF-alpha -308 SPN

was ranked at number 12 of 391 submitted papers. Further results are currently to be published:

- Fish oil induced increase in walking distance, but not ABPI, in peripheral arterial disease is dependent on both BMI and inflammatory genotype.
  J. Madden, A. Brunner, N.D. Dastur, R.M. Tan, G.B. Nash, G.E. Rainger, C.P. Shearman, P.C. Calder, R.F. Grimble

and have in part been presented at the ESPEN Congress 2006 in Istanbul:

- Influence of CD36 gene polymorphisms on the response of cardiovascular risk factors to fish oil supplementation in middle aged men.
- Polymorphisms at IL-6 -174 and TNF-α -308 and body mass index modulate the effects of fish oil supplementation on cytokine production by monocytes from healthy middle aged men.
- Altered monocyte CD44 expression in peripheral vascular disease is corrected by fish oil supplementation.
- Fish oil induced increase in walking distance, but not ABPI, in peripheral arterial disease is dependent on both BMI and inflammatory genotype.

Further analysis is under way and we expect to be able to demonstrate more interesting results and conclusions.

The aim of our work is to understand:

- which genes of the immune system are in a narrower sense linked with the development of cardiovascular disease;
- the consequences of the expression of certain alleles on monocytes producing inflammatory cytokines;
- if macrophages of patients suffering from atherosclerosis are of a special phenotype or show an extraordinary inflammatory potential which could be influenced in a beneficial way by nutritional PUFAs;
- the influence of nutrition on the phenotype of macrophages, especially on their ability to induce pro-inflammatory changes at the endothelium;
- to what extent the cardiovasculoprotective effect of fish oil is uniform or individual in respect of cytokine production, endothelial activation and leukocyte recruitment;
- if within our defined risk population the effect of such intervention can be measured by clinical endpoints.

Gaining insight into how polymorphisms in the human genome, namely of the cytokines modulate the interaction between n-3 polyunsaturated fatty acids and inflammatory process, could be an important key to understanding an individual's propensity for inflammation and the nutritional basis for major western civilization disease as is atherosclerosis at the vascular endothelium. In future this knowledge could allow reasonable prophylactic and therapeutic interventions to be aimed at the individual genotype, thus saving a lot of money and achieving much better accuracy and success within an area of considerable epidemiological relevance for public health.

Beside SoFiA my research stay in Southampton was the starting point for further smaller projects, questions and publications:

- The diagnostic validity of urinary albumin as a measure for mikrovascular leakage and inflammatory status in the assessment of efficiency of nutritional interventions in patients suffering from peripheral vascular disease.
- Genetic cytokine polymorphisms in patients with gastric cancer and HIV respectively.

  ESPEN 10/2006 Istanbul: Serum concentrations of TNF-α as a surrogate marker for malnutrition and worse quality of life in patients with gastric cancer.
- In cooperation with the Department of Diabetes and Endocrinology at the University of Hull: Correlation of insulin resistance with flow cytometrically described cytokine pattern of patients with polycystic ovarian syndrome PCOS.
- Review article about the refeeding syndrome: "Nutrition in Clinical Practice: The refeeding syndrome - Illustrative cases and guidelines for prevention and treatment". This publication, written in cooperation with Dr. Zeno Stanga, Bern; Prof. Robert F Grimble, Southampton; Prof. Alan Shenkin, Glasgow and Prof. Simon P Allison, Nottingham is currently in press and might become the basis for the European ESPEN-treatment guidelines.

I wish to thank the SFEFS for the substantial support that was given to me, allowing me to gain precious experience at a place of scientific excellence.

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