A nutrigenomic strategy to assess the physiological properties of fermented dairy products

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The 21st century will witness the mutation of nutrition research from molecular nutrition to nutritional systems biology. Indeed, nutrigenomics is already deciphering the interaction of food with human organisms and nutrigenetics is evaluating the contribution of individual genetic configurations to this interaction. Because of their cultural, nutritional, and economical importance, dairy products have always raised scientific and public debate. On one hand, a wealth of peer-reviewed publications highlights the broad physiological benefits of dairy products. On the other hand, some challenge the safety and relevance of the consumption of bovine milk by human adults. In this context, nutrigenomics provides a challenging analytical strategy to complement the information already available on these issues. The potential of nutrigenomics for dairy research is illustrated by presenting two in vivo studies, the first in humans and the second in mice, that investigate how the blood cells transcriptome responds to the ingestion of dairy products and probiotics, respectively.

The human study investigates global gene expression in blood cells of healthy human volunteers after ingestion of milk. Ingestion of milk (coagulated with glucono delta lactone to mimic the texture properties of the yogurt presented in the next section) results in the identification of circa five hundred differentially expressed genes. A kinetic analysis of the postprandial blood cell transcriptome reveals two pools of genes. A first pool is transiently down-regulated 2h after the ingestion of milk before being more strongly up-regulated after 6h. These genes almost exclusively belong to biological pathways involved in protein synthesis and mitochondrial activity. The second pool of genes is first transiently up-regulated after 2h before being more strongly down-regulated after 6h. These genes are almost exclusively involved in immunomodulatory processes, in particular apoptosis and inflammation. We hypothesize that the transient changes in genes observed at 2h are unspecific and reflect the ability of macronutrient to induce metabolic cellular stress in the human organism whereas the effects observed at 6h may be more reflective of the biological effects of specific nutrients in milk. Remarkably, almost all differentially expressed genes belonging to the Toll-like receptor (TLR) pathway are down-regulated after 6h (TLR2, TLR4, CD14, NFκB, IL8, …). An analysis of the putative transcription factors modulating gene expression further substantiates the central role of NFκB in the response of the volunteers to the ingestion of milk. Interestingly several studies, in vitro and in animal models, suggest that TLR4 may mediate pro-inflammatory properties of saturated fat by acting as a receptor for this lipid. However, the down-regulation of the genes of the TLR pathway observed after 6h in our study contrasts with these results. Our observations are not only in line with recent human nutritional trials proposing anti-inflammatory properties of dairy products but also highlight the importance of investigating these products in humans in the context of their complex food matrix.

In the same human study we have also compared the postprandial blood cell transcriptomes of volunteers having ingested milk and a fermented dairy product (i.e. yogurt). This comparison reveals several key findings: Firstly, both transcriptomes are globally similar as most of the genes, which are differentially expressed after the ingestion of milk, follow the same kinetic pattern of expression after ingestion of yogurt. This observation is in line with the similar composition in macronutrients of both dairy products; Secondly, a quantitative correlation analysis of the complete set of genes modulated by the ingestion of milk and yogurt suggests that fermentation broadens the transcriptomic response of the blood cells in the volunteers, in line with the more complex molecular and cellular composition of the fermented dairy product; Thirdly, single genes can be identified that respond differentially to the ingestion of milk and yogurt, suggesting that such genes may be selected as biomarkers to monitor the effect of milk fermentation on human physiology.

The second study in mice investigates whether transcriptomics can be used to evaluate the potential of probiotics in inhibiting infections with pathogenic bacteria. To this end, we have measured the blood cell transcriptome of mice in a gastric co-inoculation model composed of the enterohemorrhagic strain *Escherichia coli* 0157:H7 and a *Lactobacillus gasseri* strain that has demonstrated probiotic properties in vitro and in animal models. The experimental conditions, including the selected mice strain and the dose of *E. coli* used for the gastric infection, are such that classical clinical endpoints (e.g. diarrhea and hematology) and immunological endpoints (e.g. macrophage activation) do not reveal a pathogenic action of *E. coli* 0157:H7 on the mice. However, statistical analyses of the blood cell transcriptomic profiles, using principal component analysis and hierarchical clustering, clearly differentiate between the various treatment groups (e.g. control mice, mice inoculated with *E. coli* only, mice inoculated with *L. gasseri* only, mice co-inoculated with *E. coli* and *L. gasseri*). In addition, the blood cell transcriptome of mice co-inoculated with *E. coli* and *L. gasseri* is quantitatively closer to the transcriptome of the control group of mice than to the transcriptome of the *E. coli* group, suggesting an inhibitory action of *L. gasseri* in this sub-clinical *E. coli* infection model. Finally, an analysis of the pathways modulated by the differentially expressed genes allows a discussion on the mechanisms of action of the two bacterial strains that involves cell adhesion as well as metabolic and immune regulation.

Taken together, our studies suggest that the blood cell transcriptome can be used as a source of biomarkers to evaluate the consequence of the fermentation of milk on human physiology as well as the therapeutic or preventive potential of probiotic strains. This analytical strategy may eventually lead to the selection of bacteria transforming milk into products with enhanced nutritional or health properties. In that context, a research initiative was recently granted by the Swiss National Foundation and the Nano-Tera program, to develop an integrated lab-on-a-chip platform that investigates the effects of food ingestion by humans (http://www.nano-tera.ch/projects/403.php). The core of this system is an integrated chip, the NutriChip, which is an artificial and miniaturized gastro-intestinal tract that will be able to probe the health potential of dairy food samples, using a set of protein biomarkers identified through the above in vivo gene expression studies.