Introduction
The increasing market appeal of foods that have health benefits beyond the nutritional is defining market strategies for many new food products. Some examples of functional foods include margarine with added cholesterol-lowering phytoestrogens, breads fortified with fish oil and more recently, palatable soy flour. Justifying claims of functionality is difficult, given the nature of the substances, route of delivery and mechanism of action. In the majority of cases, functional agents are applied in the treatment of chronic conditions or as preventative against future illness and so measuring efficacy may defy the use of traditional measures.

Analysis of samples requires a multi-disciplinary approach that covers the food from raw materials through to the finished product. As with all therapeutic substances, functional foods are increasingly scrutinised using in vivo studies that explore bioavailability, therapeutic effect, toxicology and metabolism of the active substances. Here, we discuss two functional additives and their metabolic pathways to illustrate the multi-disciplinary requirements to establish a causal chain in the application of the functional approach.

Unsaturated Fish Oils
Fish oils are widely acknowledged to possess anti-inflammatory and anti-oxidant activity but are also known to contribute to neural development of infants. Globally, fish oil is included in mainstream products such as bread, beverages, health supplements and infant formula. While the ω-3 fatty acids are generally acknowledged as the functional agent, uncertainty remains in ascribing the particularly high activity of oil from some species to specific (and unusual) compounds or to bioavailability under normal dietary and extreme conditions.

One species that has reputed activity as an anti-inflammatory agent is the Green-Lipped Mussel (Perna canaliculus). Analysis of the isolated fatty acid fraction (Figure 1) shows that the main oils present are eicosapentanoic acid (C20:5), and docosahexanoic acid (C22:6). The isolation of pure fatty acids is generally acknowledged as the ω-3 fatty acids required an understanding of the metabolic pathways for these functional components (Figure 3). The selective use of both COX and LTB4 generating assays for various fatty fractions processed from the mussel and isolated pure fatty acids suggests that overall free fatty acid bioavailability and not highly active minor components are responsible for the activity of the species (McPhee et al. 2001, 2003).

Implications of the Study
The identification of specific compositions of total lipid as contributing to the activity of the oil poses questions in the study of processed fish oils or fish products incorporated into foods. While our investigation examined both free fatty acids and freeze-dried fish powder as a health food supplement, our results cannot be simply extrapolated to all functional examples without first correcting the data for bioavailability and dose, the changed distribution of lipid classes and a change in the distribution of individual fatty acids within each class on processing.

Analysis of Functional Foods: The Fine Line Between Nutritional and Therapeutic Effects

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Introduction

Unsaturated Fish Oils

Figure 2. The functional components of fish oil, identified using GC resolution on a BPX70 column.

Figure 3. The arachidonic acid cascade and the point at which fish oil can exert its competitive inhibitory effects on the lipooxygenase and cyclooxygenase pathways.

Implications of the Study

The significant difference between thiamine cation and thiamine thiol is the likely ability of the latter to cross the blood brain barrier. Under conditions of hypervitaminosis, leading to the release of abnormally high concentrations of thiamine thiol, it is possible to propose a benzodiazepine like activity arising from the reaction of the thiol with amines (adenosine) or alternatively with central receptor proteins. Using this hypothesis, the metabolic fate of hyper-thiaminemia or following the intravenous administration of thiamine prodrugs such as thiamine disulphide (Figure 4) may be derived by derivation and GC/MS analysis of the acetylated extract on a BPX5 column (Figure 5) or by analysis of the SPE fraction directly by LC/MS-MS analysis in both positive and negative ion modes in a methanol ammonium acetate mobile phase buffered to pH 8 with ammonia. Analysis revealed that both treatments give rise to abnormally high concentrations of thiaminethiol (the monomeric form of thiamine disulphide) in the urine. The identity of the compound was confirmed by independent synthesis.

Figure 4. The active forms of Vitamin B1, thiamine and thiamine disulphide. The analysis of the vitamin and prodrugs can be carried out by HPLC using a Protein-Pak C18 or LiChroPrep column, with UV detection at 210 and 254 nm, respectively.

Figure 5. GC chromatogram of analytically obtained thiamine as hydrochloride, and intravenously administered thiamine disulphide, analysed using a BPX5 column.

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Analysis of minor components show that thiamine thiol has a propensity for further intramolecular and extramolecular reaction. The formation of the so-called diazepine artefacts was found to be a process driven by either GC injector port temperature or alternatively, as a thermodynamically preferred process during fragmentation in the mass spectrometer using GC or direct inlet. The native sulphide could be captured in negative ion LC/MS using basic conditions. Discounting the diazepine compounds as physiologically available substances suggests that thiamine thiol or one of its reaction products is responsible for the anti-anxiolytic effects of thiamine.

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One species that has reputed activity as an anti-inflammatory agent is the Green-Lipped Mussel (Perna canaliculus). Analysis of the isolated fatty acid fraction (Figure 1) shows that the main oils present are eicosapentanoic acid (C20:5), and docosahexanoic acid (C22:6) (Figure 2). Significant to the elucidation of a functional mechanism, several non-branched fatty acids, the major one being isomeric with eicosapentanoic acid (C20:5), and docosahexanoic acid (C22:6) isolated fatty acid fraction (Figure 1) shows that the main oils present

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