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Invited Commentary

Impact of phytosterols on mitochondrial functions

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Phytosterols are structurally related to cholesterol and are mainly C28 and C29 carbon steroid alcohols[1]. Plant sterols, also named phytosterols, are integral components of the membrane lipid bilayer of plant cells[2]. Unlike animal systems in which cholesterol is most often the single final product of sterol synthesis, each plant species has its own characteristic distribution of phytosterols, with the three most common phytosterols in nature being β-sitosterol, campesterol and stigmasterol[3]. In addition to the free sterol form, phytosterols are also found in the form of conjugates, particularly fatty acyl sterol esters.

In humans, phytosterol absorption is considerably less than that of cholesterol[4]. Some investigations support that phytosterols decrease cholesterol absorption, and thus reduce circulating concentrations of cholesterol[5]. Indeed, in the intestine, phytosterols compete with cholesterol[6], leading to reduced cholesterol absorption and, as a consequence, to a lower plasma LDL-cholesterol concentration[7]. In addition, phytosterols appear not only to play an important role in the regulation of CVD but also to exhibit anti-cancer properties[8,9]. The major currently identified and well-recognised side effects associated with the consumption of phytosterols are that they reduce the blood concentrations of fat-soluble vitamins, such as vitamins A, D, E and K[10,11], and that they favour an increase in plasma phytostanols[12], resulting from phytosterol auto-oxidation[13,14], which have been described to trigger cell death on different cell types when used at elevated concentrations[15–17]. Based on their established and putative beneficial effects, plant sterols have been added to various food matrices, including juices, non-fat beverages, milk and yogurt, margarine, and cheese[18], which are among the most prominent examples of a set of foods designated as 'functional foods'. The drawback of functional foods is that they can provide nutrients at levels above and beyond existing recommended intakes, and that they are inconsistent with the definition of physiological requirement[19]. As relatively little is known about the effects of chronic consumption of functional foods enriched in phytosterols, their impact on cellular organelles, especially mitochondria and peroxisomes playing major roles in glucose and/or lipid metabolism[20,21], cannot be excluded and needs to be clarified.

Currently, whereas only a few data are available on the effects of phytosterols on mitochondria, a number of investigations have supported that this organelle can constitute a potential direct or indirect target of phytosterols. Thus, stigmasterol can alter the voltage-dependence of the voltage-dependent anion-selective channel purified from the mitochondria of bean seeds (*Phaseolus coccineus*)[22]. However, on isolated brain mitochondria, stigmasterol and β-sitosterol had no effect on mitochondrial functions studied at concentrations up to 100 μmol/mg protein[23]. On the other hand, β-sitosterol favours an apoptotic mode of cell death associated with mitochondrial modifications, including a cytosolic release of cytochrome *c* in HT116 human colon cancer cells[24], and conferred a radioprotective effect on thymocytes by acting on the maintenance of mitochondrial membrane stability[25]. At the mitochondrial level, some major enzymes involved in cholesterol transport and metabolism, such as the gonadal steroidogenic acute regulatory protein (StAR) and the hepatic mitochondrial sterol 27-hydroxylase (CYP27A1), participating in the degradation of cholesterol to bile acids can be altered by sitosterol[26,27]. Thus, *in vivo* implants of β-sitosterol in male goldfish (*Carassius auratus*) not only cause reductions of reactive cholesterol pools in mitochondria isolated from gonads but also decrease the expression of gonadal StAR, a transport protein that regulates cholesterol transfer within the mitochondria, which is the rate-limiting step in the production of steroid hormones[27,28]. In addition, analysis of Lineweaver–Burk double reciprocal plots of sterol 27-hydroxylase activities on human liver extracts (where mitochondrial sterol 27-hydroxylase activities were measured with increasing concentrations of the cholesterol substrate, in the absence and presence of 100 and 300 μM-sitosterol) revealed that sitosterol inhibited mitochondrial sterol 27-hydroxylase activity up to 50 % by a competitive mechanism[26]. Moreover, the *in vitro* investigation by Danesi *et al.*[29], published in this issue of the *British Journal of Nutrition*, on supplemented rat cardiomyocytes with different concentrations of a phytosterol mixture (mainly containing sitosterol, campesterol and stigmasterol) within the range of plasma concentrations considered effective for cholesterol lowering[30], clearly shows that phytosterols used in these conditions did not induce apoptosis, but rather favour a reduction in metabolic activity (measured as 3-(4,5-dimethyldiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) conversion) and a slowing down of cell growth. The lower MTT conversion and the similar lactate dehydrogenase release

suggest that phytosterols more efficiently target mitochondria than plasma membrane integrity, a possibility that cannot be excluded.

Thus, based on currently published data obtained by different laboratories, there is some evidence that mitochondria could be a potential direct or indirect target of phytosterols, and that these can trigger some mitochondrial dysfunctions even at concentrations considered effective for cholesterol lowering. Therefore, as mitochondria are a major cellular organelle involved in energy production, glucose and lipid metabolism, it is important to identify, in a metabolic context, the impact of phytosterols on this organelle in terms of ATP production and fatty acid β-oxidation, especially in subjects regularly eating and/or drinking 'functional foods' supplemented with phytosterols.

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