British Journal of Nutrition (2004), **91**, 3–4
© The Author 2004
DOI: 10.1079/BJN20031048

https://doi.org/10.1079/BJN20031048 Published online by Cambridge University Press

Invited commentary

Sterol autoxidation: from phytosterols to oxyphytosterols

Sterols are important structural components of cell and organelle membranes of higher organisms: they regulate membrane fluidity and permeability as well as membrane-associated metabolic processes (Kritchevsky, 1997). Whereas mammalian and fungal cells generally contain one major sterol, cholesterol and ergosterol respectively, plants have a characteristically complex sterol mixture of cholesterol analogues termed phytosterols (Bean, 1973). Sterols are the precursors of a vast array of compounds involved in cellular and developmental processes in animals (e.g. steroid hormones and bile acids), fungi (e.g. ecdysteroids, antheridiol and oogoniol) and higher plants (e.g. brassinosteroids) (Hartmann, 1996; Bishop & Yokota, 2001). Plant sterols resemble cholesterol structurally in that they all have a steroid nucleus, a 3β-hydroxy group, and a double bond between C atoms five and six. The major differences are in side-chain substitution and/or saturation (Fig. 1) (Fieser & Fieser, 1959). The principle plant sterols are β-sitosterol (24α-ethylcholesterol), which comprises 45–95 % of the total sterol present in plants, campesterol (24α-methylcholesterol), which may account for 30 % of the total sterols of seed oils, and stigmasterol (Δ^{22},24α-ethylcholesterol), which may account for as much as 25 % of the total sterol of seed oils (Weihrauch & Gardner, 1978).

An oxysterol is a sterol containing one or more additional oxygen functionalities and is formed as a result of oxidation of cholesterol, some of its immediate precursors or of its analogues. Oxysterols have been ascribed a number of important roles in connection with cholesterol turnover, atherosclerosis, apoptosis, necrosis, carcinogenisis, inflammation, immunosuppression and the development of gallstones (Björkhem & Diczfalusy, 2002). In the case of phytosterols, the terminology for oxidized plant sterols, termed oxyphytosterols, is similar to that described for oxysterols. Oxysterols and oxyphytosterols, as present in the human body, may be derived from absorption of oxidized sterols present in the food, as well as from endogenous origin. The cytotoxicity of oxyphytosterols has been examined by studies in a cultured-derived macrophage cell line, C57BL/6. Results indicate that the oxides of β-sitosterol and campesterol have similar patterns of toxicity compared with oxysterols, as indicated by LDL leakage, cell viability and mitochondria dehydrogenase activity (Adcox *et al.* 2001).

Oxysterols can be formed by physical processes such as heating and radiation, by non-enzymatic processes involving reactive oxygen and free radical species (Smith, 1981), or enzymatically, by specific cytochrome P450 monooxygenases (Russell & Setchell, 1992). The B ring

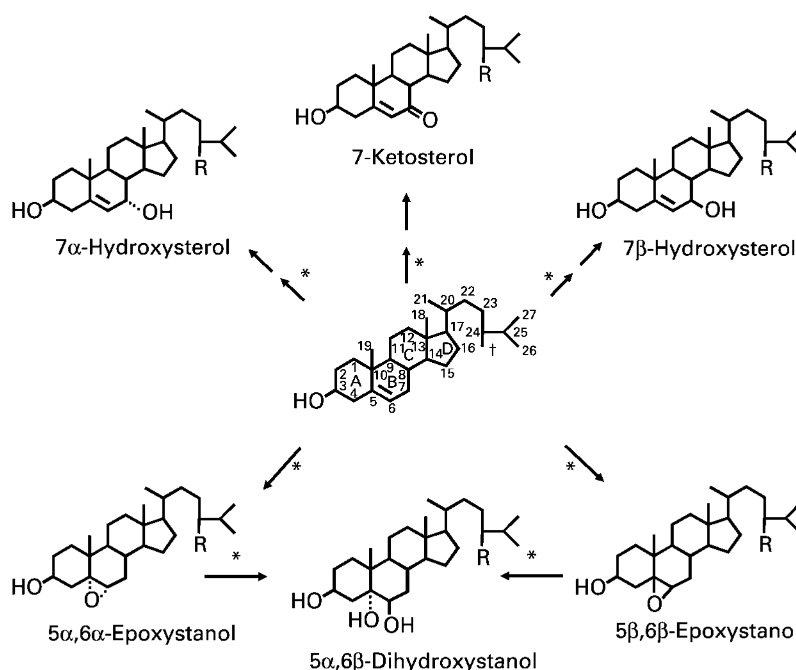

Fig. 1. Sterol autoxidation products. R: H, cholesterol; CH_3, campesterol; C_2H_5, sitosterol; C_2H_5, Δ22, stigmasterol. *, Radical oxygen species: O_2, O_3, $E = h \cdot \nu$, T.

Δ5 unsaturation and positions allylic to it are particularly susceptible to oxidative attack (Fig. 1). The primary products of cholesterol and phytosterol autoxidation are instable peroxy radicals, which are finally reduced to hydroxyl groups. The epoxides and their common hydration products, e.g. 5α-cholestane-3β,5,6β-triol from cholesterol or, in a common term, 5α,6β-dihydroxystanols from sterols, are exclusively formed by sterol autoxidation (Fig. 1).

Oxysterols and oxyphytosterols are most often present in the proximity of their parent compounds cholesterol and phytosterols. This constitutes a large risk of quantitation artifacts, as even minor sterol autoxidation during sample storage and processing would yield substantial elevations of oxysterol and oxyphytosterol concentrations. During the last few years various techniques have evolved to minimize this potential error source: (1) sample processing and purification under an inert atmosphere; (2) addition of chelators that bind transition metal ions, thereby preventing radical initiation reactions; (3) addition of antioxidants that quench free radical reactions; (4) addition of isotope-labelled sterols in order to trace and quantitate autoxidation during sample processing (Dzeletovic *et al.* 1995; Guardiola *et al.* 2002). The development of GLC using a fused-silica capillary allows the use of highly sensitive detection systems, such as selective ion monitoring–MS. Advanced liquid chromatography combined with MS increases the sensitivity and specificity of normal HPLC with electrochemical or UV-absorbance detectors.

Recently, we found that oxyphytosterols were present in markedly elevated concentrations in serum from sitosterolaemic patients, while the concentrations of serum oxyphytosterols in healthy control subjects was below the limit of detection (Plat *et al.* 2001). Patients with sitosteroleamia hyperabsorb and retain all sterols, including plant and shellfish sterols from the intestine; this is caused by mutations in two tandem ATP-binding cassette transporter (ABC) genes, ABCG5 and ABCG8 (Berge *et al.* 2000; Lee *et al.* 2001). In the present issue of *British Journal of Nutrition*, Grandgirard *et al.* (2004) describe the existence of noticeable quantities of β-epoxysitostanol and sitostanetriol in the sera of healthy subjects, and also trace levels of α-epoxysitostanol, campestanetriol and 7-ketositosterol.

The development of accurate and sensitive methods for qualitative and quantitative analyses of oxysterols and oxyphytosterols in food, dietary products and biological samples has become a new challenge for basic investigations in agriculture science and nutritional and clinical research.

Dieter Lütjohann
Department of Clinical Pharmacology
University of Bonn
Sigmund-Freud-Str. 25
D-53105 Bonn
Germany
d.luetjohann@uni-bonn.de

References

Adcox C, Boyd L, Oehrl L, Allen J & Fenner G (2001) Comparative effects of phytosterol oxides and cholesterol oxides in cultured macrophage-derived cell lines. *J Agric Food Chem* **49**, 2090–2095.

Bean GA (1973) Phytosterols. *Adv Lipid Res* **11**, 193–218.

Berge KE, Tian H, Graf GA, *et al.* (2000) Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. *Science* **290**, 1771–1775.

Bishop GJ & Yokota T (2001) Plants steroid hormones, brassinosteroids: current highlights of molecular aspects on their synthesis/metabolism, transport, perception and response. *Plant Cell Physiol* **42**, 114–120.

Björkhem I & Diczfalusy U (2002) Oxysterols: friends, foes, or just fellow passengers? *Arterioscler Thromb Vasc Biol* **22**, 734–742.

Dzeletovic S, Breuer O, Lund E & Diczfalusy U (1995) Determination of cholesterol oxidation products in human plasma by isotope dilution-mass spectrometry. *Anal Biochem* **225**, 73–80.

Fieser LF & Fieser M (1959) *Steroids*. New York: Reinhold Publishing Corporation.

Hartmann MA (1996) Plant sterols and the membrane environment. *Trends in Plant Sci* **3**, 170–174.

Grandgirard A, Martine L, Demaison L, *et al.* (2004) Oxyphytosterols are present in plasma of healthy human subjects. *Br J Nutr* **91**, 101–106.

Guardiola F, Codony R & Savage GP (editors) (2002) *Cholesterol and Phytosterol Oxidation Products. Analysis, Occurrence, and Biological Effects*. Champaign, IL: AOAC Press.

Kritchevsky D (1997) Phytosterols. *Adv Exp Med Biol* **427**, 235–243.

Lee MH, Lu K, Hazard S, *et al.* (2001) Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. *Nat Genetics* **27**, 79–83.

Plat J, Brzezinka H, Lütjohann D, Mensink R & von Bergmann K (2001) Oxidized plant sterols in human serum and lipid infusions as measured by combined gas-liquid chromatography-mass spectrometry. *J Lipid Res* **42**, 2030–2038.

Russell DW & Setchell KD (1992) Bile acid biosynthesis. *Biochemistry* **31**, 4737–4749.

Smith LL (1981) *Cholesterol Autoxidation*. New York: Plenum Press.

Weihrauch JL & Gardner JM (1978) Sterol content of foods of plant origin. *J Am Diet Assoc* **73**, 39–47.

https://doi.org/10.1079/BJN20031048 Published online by Cambridge University Press