



## European network for oxysterol research (ENOR): 10 th anniversary

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The European Network for Oxysterol Research (ENOR ; <https://www.oxysterols.net/>) was created by Dr G erard Lizard (Inserm / University of Burgundy, Dijon, France) and Prof Luigi Iuliano (Sapienza University of Rome, Rome, Italy) after having gathered in 2010 in Munich a large number of European leaders involved in research on oxysterols and phytosterols during a joint meeting LipidomicNet and ENOR (organizers: L Iuliano, M Lagarde, G Lizard, G Schmitz, M Wakelam). Since then, and until 2019, an annual symposium has been organized by ENOR in different European university cities gathering scientists from different backgrounds and many students. Symposia have been held successively in Rome - Italy (2011; local organizer: L Iuliano), Dijon - France (2012; local organizers: L Bretillon, G Lizard), Swansea - UK (2013; local organizer: WJ Griffiths), Coimbra - Portugal (2014; local organizer: ML S  E Melo), Bonn - Germany (2015; local organizer: D L tjohann), Paris - France (2016; local organizer: C Massaad), Brussels - Belgium (2017; local organizer: G Muccioli), Bologna - Italy (2018; local organizers: MT Rodriguez Estrada and V Cardenia), Edinburgh - Scotland (2019; local organizers: R Andrew, M Dixon and N Homer). In 2020, the ENOR meeting has been postponed due to the COVID-19 pandemic.

The aim of the ENOR is to promote interactions between research groups, to enroll young investigators in the field, and to stimulate novel researches on oxysterols and phytosterols, on the identification of new sterol metabolites or derivatives, on the characterization of the biological, physiological, and patho-physiological properties of these molecules, and on innovative therapies against oxysterol-associated diseases [1,2]. ENOR is a self-promoting and self-sustaining organization, which is opened to research groups worldwide. ENOR members exchange ideas and share data during the annual meetings, and by conventional internet-based tools. ENOR has about 100 members, from Australia, Belgium, Brazil, Czech Republic, Finland, India, Ireland, Israel, Italy, Japan, Finland, France, Germany, Lebanon, Lithuania, Morocco, Netherlands, Poland, Portugal, Russia, Serbia, Slovenia, Spain, Switzerland, Sweden, Tunisia, Turkey and United Kingdom. Scientists from USA also regularly participate to the ENOR symposia.

Ten years after its creation, we can assume that ENOR has contributed a lot to maintain, stimulate and promote research on oxysterols and phytosterols, which are still little known molecules despite their important physiological activities and their implications in many diseases [3–5].

As a reminder, cholesterol (C<sub>27</sub>H<sub>46</sub>O ; molecular weight : 386.65 ; chemical names : 3 -hydroxy-5-cholestene or 5-Cholesten-3 -ol; PubChem CID 5997), initially called "cholesterine", was discovered in 1815 by the French chemist Eug ne Chevreul, but it was only in 1884 that Emile Littr  introduced the term "cholest rol" in his medical dictionary [6]. The name of cholesterol originates from the ancient Greek *chole* (bile) and *stereos* (solid), because it has been initially discovered in its solid form in galstones in 1758 by the French chemist Fran ois Poulletier de la Salle [6]. In the course of the 20th century, numerous studies on cholesterol have been carried out, leading to four Nobel prizes : in 1928, Adolf Windaus for his research on sterols ; in 1964, Konrad Bloch and Feodor Lynen for discovering the mechanism of cholesterol and fatty acid metabolism ; in 1975, John Cornforth for his studies on enzymes involved in cholesterol biosynthesis ; in 1985, Michael S Brown and John L Goldstein for their works on cholesterol metabolism and Low Density Lipoprotein (LDL) receptors [6]. These works have led to a better understanding of cholesterol by elucidating its metabolism, establishing the role of cholesterol in cardiovascular diseases [7], discovering the lipoproteins that transport cholesterol and leading to cholesterol-lowering drugs: the statins ; the first statin, compactin, was described by Dr. A. Endo and colleagues (Sankyo Co, Japan) in the 1980s [8,9]. Cholesterol, which is heterogeneously distributed, with only 0.5–1 % of total cell cholesterol present in the endoplasmic reticulum and 60–80 % in the plasma membrane, is involved in cell signalling and is a precursor of steroid hormones and bile acids [10]. Thus, if cholesterol excesses are deleterious, cholesterol is a molecule essential to animal life.

It is also now well established that cholesterol can give rise to oxygenation products called oxysterols. These molecules were described by Lifschutz in 1913, who called them oxysterols [6,11]. It was not until the 1970 s–1980 s that AA Kandutsch and his colleagues called

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them oxysterols [12]. Since the 1970s, thanks to the initial research of AA Kandutsch but also of B Craste de Paulet and their collaborators, the works on oxysterols have continuously increased [12,13]. Oxysterols, which are part of the oxygenation products of cholesterol; they are endogenously produced by a variety of cells via enzymatic activities (monooxygenases, oxidoreductases, hydrolases, transferases), auto-oxidation (radical and non-radical processes), or both [14]. Several major oxysterols also arise as intermediates in the pathways converting cholesterol to bile acids or steroid hormones [2,11]. As a result of cholesterol oxidation, polar groups (hydroxy, keto, hydroperoxy, epoxy, or carboxyl) are introduced to the cholesterol molecule. Oxysterols poly-oxygenated and conjugated can give rise to an expanding family of structurally diversified steroids [15]. Oxysterols are involved in the control of metabolism, proliferation, differentiation and cell death [2,16]. Some of them are also involved in oxidative stress and inflammation [4,17]. Therefore, these molecules display specific physiological roles according to their chemical structure. Variation of the level of some oxysterols has been linked to several diseases including age-related diseases: cardiovascular diseases, eye diseases (age-related macular degeneration, cataract) neurodegenerative diseases (Alzheimer's disease, Huntington's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis), osteoporosis and cancers [3]. Some oxysterols involved in these diseases have been characterized as specific ligands and modulators of nuclear or membranous receptors controlling specific signalling pathways [18]. Indeed, the specific mechanisms involved in oxysterols biosynthesis and metabolism, as well as oxysterol - effectors constitute potential promising pharmacological targets for several diseases. There is also recent evidence that several oxysterols have antiviral activities against both enveloped and non-enveloped viruses [19,20] and are involved in specific stages of COVID-19 infectivity [21].

In general, cholesterol and oxysterols can be considered as almost specific molecules of animal cells since little cholesterol is present in plant cells [22,23]. However, contrary to popular belief, the plants contain cholesterol both free and esterified in low amount, and cholesterol occurs as a component of plant membranes and as part of the surface lipids of leaves where it is sometimes the major sterol [23]. Consequently, cholesterol can be present in significant quantities in products of vegetable origin such as oils (corn oil : 55 mg/kg ; Canola oil : 53 mg/kg ; cotton seed oil : 45 mg/kg) [23]. In olive oil, which is widely used in the Mediterranean diet, cholesterol is however present at very low amount : 0.5–2 mg/kg [23,24]. On the other hand, all plants synthesize and contain phytosterols which are plant sterols distinguishable from cholesterol by the presence of a methyl or ethyl group on carbon 24 ; the most common phytosterols in the human diet are  $\beta$ -sitosterol, campesterol and stigmasterol [25]. Phytosterols are known to have several bioactive properties : they reduce intestinal cholesterol absorption which alleviates blood LDL-cholesterol and several data support that they can contribute to prevent cardiovascular problems whereas it is also well established that high phytosterols consumption can favor sitosterolemia [26–28]. Case-control studies indicate that high dietary intake phytosterol / phytostanol were associated with reduced risks of several cancers [29,30]. In addition, some phytosterol oxidation products (oxyphytosterols) exhibit pro-atherogenic properties, cytotoxicity, oxidative stress, apoptosis, and proinflammatory properties [28]. Thus, the pathophysiological impact of dietary phytosterols and their metabolites deserves further studies.

The numerous works on oxysterols and phytosterols underline the dynamics of the research on these molecules. Beyond its scientific interest and because of the numerous diseases among which oxysterols and phytosterols can have deleterious or beneficial effects, the ENOR has an international public health interest by gathering high level scientists working in this field. Despite the COVID-19 pandemic, members of the ENOR remained connected during the year 2020 and continued to work

together as evidenced by this special issue entitled 'ENOR : 10th anniversary'.

As activities of oxysterols on the immune system and viral infections have been previously reported, several works have emerged showing new activities of these molecules in this field [19,31]. The review by Imen Ghzaïel et al. describes the effects of 7-ketocholesterol on viral infections and its potential contribution in the pathophysiology of COVID-19 [32]. The results of Lucio Boglione et al., from Dr Valerio Leoni's group, show a marked reduction of 25-hydroxycholesterol (25-OHC) and 27-hydroxycholesterol (27-OHC) plasma levels in all active chronic hepatitis B (CHB) virus recruited patients, while the plasma values observed in inactive carriers remained within the physiological range this study points to 27-OHC as a good candidate biomarker to differentiate active and inactive CHB status [33]. Several works also underline the importance of oxysterols on the metabolism and their implication in many diseases. J Abdel-Khalik from Swansea University (Prof WJ Griffiths, Dr Y Wang) shows the production of bile acids from 7-dehydrocholesterol in the plasma of patients with Smith-Lemli-Opitz syndrome and describes that intermediates in the pathway, 25-hydroxy-7-oxocholesterol, (25R)26-hydroxy-7-oxocholesterol, 3 $\beta$ -hydroxy-7-oxocholest-5-en-(25R)26-oic acid and the analogous 7 $\beta$ -hydroxysterols are modulators of the activity of Smoothed (Smo), an oncoprotein that mediates Hedgehog (Hh) signalling across membranes during embryogenesis and in the regeneration of postembryonic tissue [34]. T Jahn shows the importance of cerebrospinal fluid (CSF) levels of several non-cholesterol sterols and oxysterols to Alzheimer's disease and core Alzheimer's disease biomarkers. The plant sterols campesterol and sitosterol appear to be involved in tau pathology and neurodegeneration. CSF desmosterol level indicates central nervous system cholesterol synthesis and might be of relevance for clinical disease severity [35]. Dr A Vejux reports that different lipids including fatty acids, fatty acid peroxidation products, phospholipids as well as oxidized derivatives of cholesterol (oxysterols) could constitute biomarkers providing information on the form of multiple sclerosis, the outcome of the disease and the answer to treatment [36]. On another hand, the edible Asian brown alga *Sargassum fusiforme* (Hijiki) contains high amounts of oxysterols such as (3 $\beta$ , 24 $\xi$ ) - stigmasterol-5, 28-dien-3, 24-diol (24[R, S] - saringosterol) that is a potent liver X receptor agonists. In his work, K Vanbrabant describes the procurement, purification and stability of 24(R,S)-Saringosterol ; K Vanbrabant shows that brown algae *Undaria pinnatifida* harvested in February and *Sargassum muticum* harvested in October contained the highest amounts of 24(R, S)-saringosterol and its precursor fucosterol, higher than *Sargassum fusiforme*, while *Ascophyllum nodosum* and *Fucus vesiculosus* and *Fucus serratus* contained amounts of 24(R, S)-saringosterol comparable to *Sargassum fusiforme* ; the amount of 24(R, S)-saringosterol in the brown seaweeds can be modulated by light [37]. Finally, the work of DJ Morris, team of Dr Alex Odermatt, brings new information on the modulation of 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -HSD1) functions by the cloud of endogenous metabolites in a local microenvironment with potential consequences on the treatment of hypertension [38].

## Acknowledgement

We acknowledge all colleagues who agreed to contribute to this special issue of 'Journal of Steroid Biochemistry & Molecular Biology' either as authors or referees. We hope that this issue will stimulate further developments in the area.

## References

- [1] P. de Medina, K. Diallo, E. Huc-Claustre, M. Attia, R. Soulès, S. Silvente-Poirot, et al., The 5,6-epoxycholesterol metabolic pathway in breast cancer: emergence of new pharmacological targets, *Br. J. Pharmacol.* 178 (2021) 3248–3260.
- [2] V. Mutemberezi, O. Guillemot-Legrès, G.G. Muccioli, Oxysterols: from cholesterol metabolites to key mediators, *Prog. Lipid Res.* 64 (2016) 152–169.
- [3] A. Zarrouk, A. Vejux, J. Mackrill, Y. O'Callaghan, M. Hammami, N. O'Brien, et al.,

- Involvement of oxysterols in age-related diseases and ageing processes, *Ageing Res. Rev.* 18 (2014) 148–162.
- [4] G. Testa, D. Rossin, G. Poli, F. Biasi, G. Leonarduzzi, Implication of oxysterols in chronic inflammatory human diseases, *Biochimie*. 153 (2018) 220–231.
- [5] S. Silvente-Poirot, F. Dalenc, M. Poirot, The effects of cholesterol-derived oncometabolites on nuclear receptor function in cancer, *Cancer Res.* 78 (2018) 4803–4808.
- [6] C. Massaad, L. Iuliano, G. Lizard, Oxysterols and phytosterols in human health, *Chem. Phys. Lipids* 207 (2017) 49–50.
- [7] C. Andersson, A.D. Johnson, E.J. Benjamin, D. Levy, R.S. Vasan, 70-year legacy of the Framingham Heart Study, *Nat. Rev. Cardiol.* 16 (2019) 687–698.
- [8] D. Steinberg, Thematic review series: the pathogenesis of atherosclerosis. An interpretive history of the cholesterol controversy, part V: the discovery of the statins and the end of the controversy, *J. Lipid Res.* 47 (2006) 1339–1351.
- [9] P.W. Siri-Tarino, R.M. Krauss, The early years of lipoprotein research: from discovery to clinical application, *J. Lipid Res.* 57 (2016) 1771–1777.
- [10] V.A. Cortes, D. Busso, A. Maiz, A. Arteaga, F. Nervi, A. Rigotti, Physiological and pathological implications of cholesterol, *Front. Biosci. (Landmark Ed)* 19 (2014) 416–428.
- [11] A.J. Brown, W. Jessup, Oxysterols: sources, cellular storage and metabolism, and new insights into their roles in cholesterol homeostasis, *Mol. Aspects Med.* 30 (2009) 111–122.
- [12] A.A. Kandutsch, H.W. Chen, H.J. Heiniger, Biological activity of some oxygenated sterols, *Science* 201 (1978) 498–501.
- [13] F. Besème, M.E. Astruc, R. Defay, B. Descomps, A. Crastes de Paulet, Characterization of oxysterol-binding protein in rat embryo fibroblasts and variations as a function of the cell cycle, *Biochim. Biophys. Acta* 886 (1986) 96–108.
- [14] C. Zerbinati, L. Iuliano, Cholesterol and related sterols autoxidation, *Free Radic. Biol. Med.* 111 (2017) 151–155.
- [15] M. Poirot, S. Silvente-Poirot, Oxysterols: an expanding family of structurally diversified bioactive steroids, *J. Steroid Biochem. Mol. Biol.* 194 (2019) 105443.
- [16] F.A. de Freitas, D. Levy, A. Zarrouk, G. Lizard, S.P. Bydlowski, Impact of oxysterols on cell death, proliferation, and differentiation induction: current status, *Cells* 10 (2021) 2301.
- [17] A. Vejux, G. Lizard, Cytotoxic effects of oxysterols associated with human diseases: induction of cell death (apoptosis and/or oncosis), oxidative and inflammatory activities, and phospholipidosis, *Mol. Aspects Med.* 30 (2009) 153–170.
- [18] Y. Wang, E. Yutuc, W.J. Griffiths, Neuro-oxysterols and neuro-sterols as ligands to nuclear receptors, GPCRs, ligand-gated ion channels and other protein receptors, *Br. J. Pharmacol.* 178 (2021) 3176–3193.
- [19] D. Lembo, V. Cagno, A. Civra, G. Poli, Oxysterols: an emerging class of broad spectrum antiviral effectors, *Mol. Aspects Med.* 49 (2016) 23–30.
- [20] H. Ohashi, F. Wang, F. Stappenbeck, K. Tsuchimoto, C. Kobayashi, W. Saso, et al., Identification of anti-severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) oxysterol derivatives in vitro, *Int. J. Mol. Sci.* 22 (2021).
- [21] S.M. Lam, C. Zhang, Z. Wang, Z. Ni, S. Zhang, S. Yang, et al., A multi-omics investigation of the composition and function of extracellular vesicles along the temporal trajectory of COVID-19, *Nat. Metab.* 3 (2021) 909–922.
- [22] P. Benveniste, Biosynthesis and accumulation of sterols, *Annu. Rev. Plant Biol.* 55 (2004) 429–457.
- [23] E.J. Behrman, V. Gopalan, Cholesterol and plants, *J. Chem. Educ.* 82 (2005) 1791.
- [24] A. Zarrouk, L. Martine, S. Grégoire, T. Nury, W. Meddeb, E. Camus, et al., Profile of fatty acids, tocopherols, phytosterols and polyphenols in mediterranean oils (argan oils, olive oils, milk thistle seed oils and nigella seed oil) and evaluation of their antioxidant and cytoprotective activities, *Curr. Pharm. Des.* 25 (2019) 1791–1805.
- [25] J.N. Valitova, A.G. Sulkarnayeva, F.V. Minibayeva, Plant sterols: diversity, biosynthesis, and physiological functions, *Biochemistry Mosc.* 81 (2016) 819–834.
- [26] S. Feng, T. Belwal, L. Li, J. Limwachiranon, X. Liu, Z. Luo, Phytosterols and their derivatives: potential health-promoting uses against lipid metabolism and associated diseases, mechanism, and safety issues, *Compr. Rev. Food Sci. Food Saf.* 19 (2020) 1243–1267.
- [27] E. Hovenkamp, I. Demonty, J. Plat, D. Lütjohann, R.P. Mensink, E.A. Trautwein, Biological effects of oxidized phytosterols: a review of the current knowledge, *Prog. Lipid Res.* 47 (2008) 37–49.
- [28] G. García-Llatas, M.T. Rodríguez-Estrada, Current and new insights on phytosterol oxides in plant sterol-enriched food, *Chem. Phys. Lipids* 164 (2011) 607–624.
- [29] G. Cioccoloni, C. Soteriou, A. Websdale, L. Wallis, M.A. Zulyniak, J.L. Thorne, Phytosterols and phytostanols and the hallmarks of cancer in model organisms: a systematic review and meta-analysis, *Crit. Rev. Food Sci. Nutr.* (2020) 1–21.
- [30] B. Salehi, C. Quispe, J. Sharifi-Rad, N. Cruz-Martins, M. Nigam, A.P. Mishra, et al., Phytosterols: from preclinical evidence to potential clinical applications, *Front. Pharmacol.* 11 (2020) 599959.
- [31] N.J. Spann, C.K. Glass, Sterols and oxysterols in immune cell function, *Nat. Immunol.* 14 (2013) 893–900.
- [32] I. Ghzaïel, K. Sassi, A. Zarrouk, T. Nury, M. Ksila, V. Leoni, et al., 7-Ketocholesterol effects on viral infections and hypothetical contribution in COVID-19, *J. Steroid Biochem. Mol. Biol.* 212 (2021) 105939.
- [33] L. Boglione, C. Caccia, A. Civra, J. Cusato, A. D'Avolio, F. Biasi, et al., Trend of 25-hydroxycholesterol and 27-hydroxycholesterol plasma levels in patients affected by active chronic hepatitis B virus infection and inactive carriers, *J. Steroid Biochem. Mol. Biol.* 210 (2021) 105854.
- [34] J. Abdel-Khalik, T. Hearn, A.L. Dickson, P.J. Crick, E. Yutuc, K. Austin-Muttitt, et al., Bile acid biosynthesis in Smith-Lemli-Opitz syndrome bypassing cholesterol: potential importance of pathway intermediates, *J. Steroid Biochem. Mol. Biol.* 206 (2021) 105794.
- [35] T. Jahn, C. Clark, A. Kerksiek, P. Lewczuk, D. Lütjohann, J. Popp, Cholesterol metabolites and plant sterols in cerebrospinal fluid are associated with Alzheimer's cerebral pathology and clinical disease progression, *J. Steroid Biochem. Mol. Biol.* 205 (2021) 105785.
- [36] A. Vejux, I. Ghzaïel, T. Nury, V. Schneider, K. Charrière, R. Sghaier, et al., Oxysterols and multiple sclerosis: physiopathology, evolutive biomarkers and therapeutic strategy, *J. Steroid Biochem. Mol. Biol.* 210 (2021) 105870.
- [37] K. Vanbrabant, D. Van Meel, A. Kerksiek, S. Friedrichs, M. Dubbeldam, M. Schepers, et al., 24(R, S)-Saringosterol - from artefact to a biological medical agent, *J. Steroid Biochem. Mol. Biol.* 212 (2021) 105942.
- [38] D.J. Morris, A.S. Brem, A. Odermatt, Modulation of 11 $\beta$ -hydroxysteroid dehydrogenase functions by the cloud of endogenous metabolites in a local microenvironment: the glycyrrhetic acid-like factor (GALF) hypothesis, *J. Steroid Biochem. Mol. Biol.* 214 (2021) 105988.