commentary

Are 15-oxygenated sterols present in the human circulation?[1]

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In this issue of the *Journal of Lipid Research*, Björkhem and colleagues publish results of an important study of the oxysterol content of plasma from patients with multiple sclerosis and of healthy controls (1). No significant differences in oxysterol levels were observed between controls and multiple sclerosis sufferers. Björkhem et al.'s data contradicts that of Farez et al. (2) who very recently claimed differences in the serum levels of 15-oxygenated sterols between healthy controls, patients with relapsing-remitting multiple sclerosis (RRMS) and secondary progressive multiple sclerosis (SPMS).

Oxysterols are oxygenated forms of cholesterol or its precursors. They are formed enzymatically and also via autoxidation during sample work-up. The most prevalent enzymatically formed oxysterols found in plasma are 4β-, 7α-, 24S-, 25-, and 26-hydroxycholesterols, and those formed by autoxidation include 6- and 7β-hydroxycholesterols and 7-oxocholesterol (3–6). Oxysterols are challenging molecules to analyze in biological media on account of their low abundance against a high background of cholesterol. Mass spectrometry is the analytical method of choice either in its GC-MS or LC-MS format (3–6). Both formats require prior sample work-up to resolve oxysterols from more abundant lipids. Levels of free oxysterols in plasma are usually in the ng/ml range and oxysterol levels determined after hydrolysis of fatty acid esters are in the 10–200 ng/ml range (3–6). Thus, the recent paper by Farez et al. (2) claiming the presence of 15-oxygenated sterols in serum of healthy controls as well as RRMS and SPMS patients at levels above 300 ng/ml has been met with great interest by the lipidomics community. More important from a clinical perspective was the discovery of "15α-hydroxycholestene" (presumably 5α-cholest-8(14)ene-3β,15α-diol, see **Fig. 1**) at higher levels in serum from SPMS patients (>1000 ng/ml) than RRMS patients or healthy controls (500 ng/ml) (2). Farez et al. (2) went on to suggest the measurement of "15α-hydroxycholestene" can be easily applied to clinical samples and used to identify patients in the progressive phase of multiple sclerosis. Unfortunately, in the paper published by Farez et al. (2), minimum analytical details were given other than reference to a GC-MS procedure used by others to measure a different oxysterol (7), and no relevant primary data was presented. Farez et al.'s (2) work represent the first claim of "15α-hydroxycholestene" in a biological system, although a potential precursor "15-ketocholestene" (presumably 3β-hydroxy-5α-cholest-8-(14)-en-15-one), also claimed by Farez et al. to be found in plasma, has been found in rat skin by Schroepfer and colleagues (8) who suggested it to be an autoxidation product of 5α-cholest-8(14)-en-3β-ol. 5α-Cholest-8(14)ene-3-β,15α-diol and 3β-hydroxy-5α-cholest-8(14)-en-15-one are, however, commercially available synthetic sterols.

Björkhem, Diczfalusy, and colleagues have been analyzing oxysterols in human circulation for many years and have not previously observed the presence of either 5α-cholest-8(14)ene-3β,15α-diol or 3β-hydroxy-5α-cholest-8(14)-en-15-one in plasma (3, 4). Inspired by Farez et al.'s paper (2), Björkhem et al. now present their results of an investigation into the plasma content of 15-oxygenetated sterols in samples from control, RRMS, and SPMS patient groups (1). In brief, using their well-established GC-MS protocol (3) Björkhem et al. failed to find 5α-cholest-8(14)-ene-3β,15α-diol in plasma at levels above their detection limit of 2 ng/ml. There were possibly trace levels of 3β-hydroxy-5α-cholest-8(14)-en-15-one in plasma at levels below 10 ng/ml but no significant difference was observed between healthy controls and patients with RRMS or SPMS. What then is the explanation for the widely differing results of these two investigations (1, 2)? One possibility is that the methods used by Björkhem et al. (1) result in a major loss of the 15-oxygenated analyte. This can be discounted, however, as recovery of added authentic standards were close to 100%. A second possibility is that 5α-cholest-8(14)ene-3β,15α-diol and 3β-hydroxy-5α-cholest-8(14)-en-15-one are present in blood in a conjugated form resistant to saponification as used by Björkhem et al. in their study, but were hydrolyzed in the method used by Farez et al. Unfortunately, Farez et al. failed to fully de-

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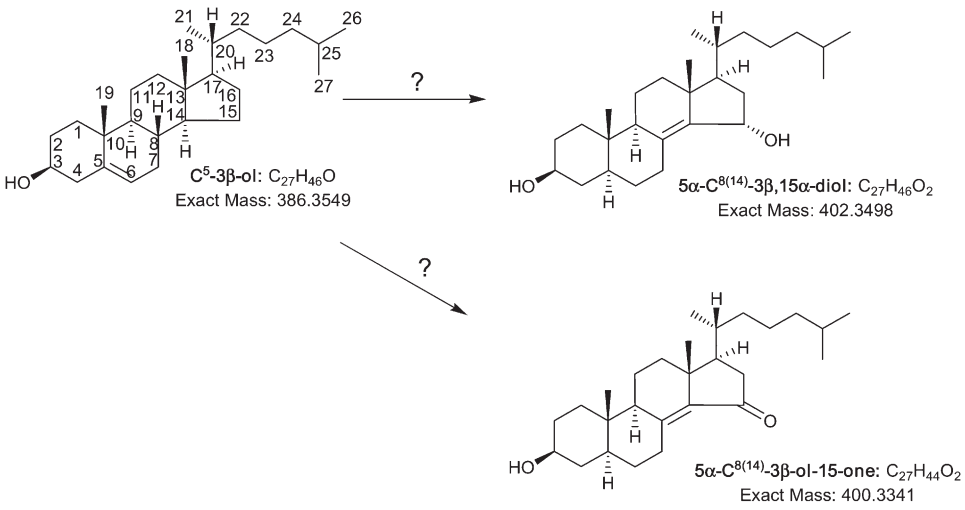

Fig. 1. Structures of cholesterol (C^5-3β-ol), 5α-cholest-8(14)ene-3β,15α-diol (5α-$C^{8(14)}$-3β,15α-diol) and 3β-hydroxy-5α-cholest-8(14)-en-15-one (5α-$C^{8(14)}$-3β-ol-15-one).

scribe their analytical protocol in their publication. The third, and in our view the most likely explanation to this puzzle, is that Farez et al. have incorrectly identified 5α-cholest-8(14)ene-3β,15α-diol and 3β-hydroxy-5α-cholest-8(14)-en-15-one in plasma, in which case their data would suggest that some unidentified lipids are increased in the circulation of patients with SPMS. It is important that these compounds are now identified.

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