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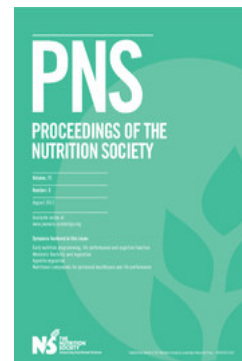
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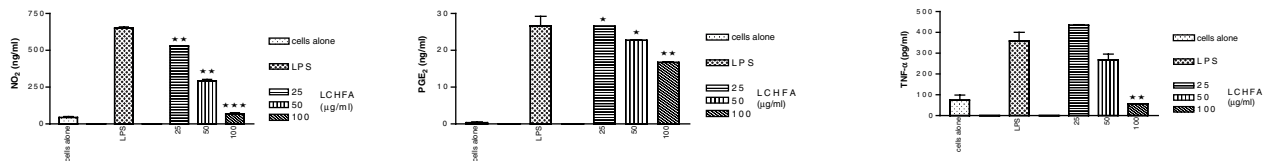
## Effect of long-chain fatty alcohols from orujo olive oil on nitric oxide and eicosanoid generation

M. A. Fernández Arche<sup>1</sup>, R. de la Puerta Vázquez<sup>1</sup>, A. Márquez Martín<sup>1</sup> and V. Ruiz-Gutierrez<sup>2</sup>

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Olive pomace oil ('orujo' oil) is an olive oil product suitable for human consumption that is traditionally produced in Spain<sup>(1)</sup>. The non-acylglycerol component of this oil is a good source of interesting minor components, e.g. triterpenes<sup>(2)</sup>, or fatty alcohols, derived from waxy materials. Tetracosanol (C<sub>24</sub>OH; 30%), hexacosanol (C<sub>26</sub>OH; 37%) and octacosanol (C<sub>28</sub>OH; 15%) are the major constituents of the long-chain fatty alcohol (LCFA) fraction isolated from orujo olive oil<sup>(3)</sup>. A similar mixture of long-chain alcohols, termed 'policosanol' and purified from waxy materials of different sources such as sugar cane, bees wax, rice bran or spinach, have shown many beneficial physiological activities<sup>(4,5)</sup>. The present study focused on the effect of LCFA isolated from orujo olive oil on NO, PGE<sub>2</sub> and TNF $\alpha$  release by a lipopolysaccharide (LPS)-stimulated murine macrophage cell line (RAW-264.7) as well as the effect on thromboxane B<sub>2</sub> (TXB<sub>2</sub>) generation by A-23187-stimulated rat peritoneal neutrophils (PMN). Nitrite (as an index of NO generation) levels were determined by a fluorometric method. PGE<sub>2</sub>, TNF $\alpha$  and TXB<sub>2</sub> production were quantified by sandwich immunoassay.

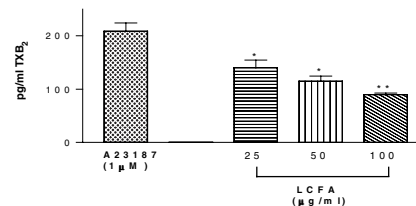
LCFA significantly and dose-dependently decreased the NO production in LPS-stimulated RAW-264.7 cell line macrophages (Fig. 1). Western-blot analysis for inducible NO synthase (iNOS) showed that NO reduction was a consequence of the 100% inhibition of iNOS expression at a dose of 100  $\mu$ g/ml (Fig. 2). By contrast, LCFA scarcely affected PGE<sub>2</sub> levels (Fig. 1). TNF $\alpha$  production was also significantly decreased by LCFA at the highest dose assayed (100  $\mu$ g/ml; Fig. 1). LCFA significantly reduced TXA<sub>2</sub> production in rat PMN stimulated with A-23187 (Fig. 3).



**Fig. 1.** Effect of LCFA on NO, PGE<sub>2</sub> and TNF $\alpha$  produced by LPS (10  $\mu$ g/ml)-stimulated RAW-264.7 murine macrophages (1  $\times$  10<sup>6</sup> cells/ml). Mean values were significantly different from those for LPS control group: \*\**P* < 0.01, \*\*\**P* < 0.001.



**Fig. 2.** Effect of LCFA subfraction on iNOS expression and densitometric analysis in RAW 264.7 cells. DEX, dexamethasone; OD, optical density.



**Fig. 3.** Effect of LCFA on TXB<sub>2</sub> produced by A-23187-stimulated rat PMN. Mean values were significantly different from the control value: \**P* < 0.05, \*\*\**P* < 0.001.

These results showed that LCFA isolated from 'orujo' oil has a protective effect on some mediators implicated in the development of inflammatory damage in these experimental models and suggest its potential value as a functional component of the olive pomace oil.

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