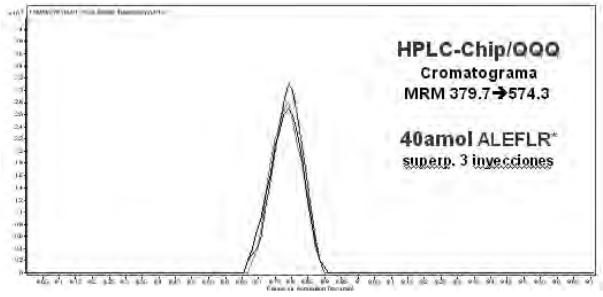

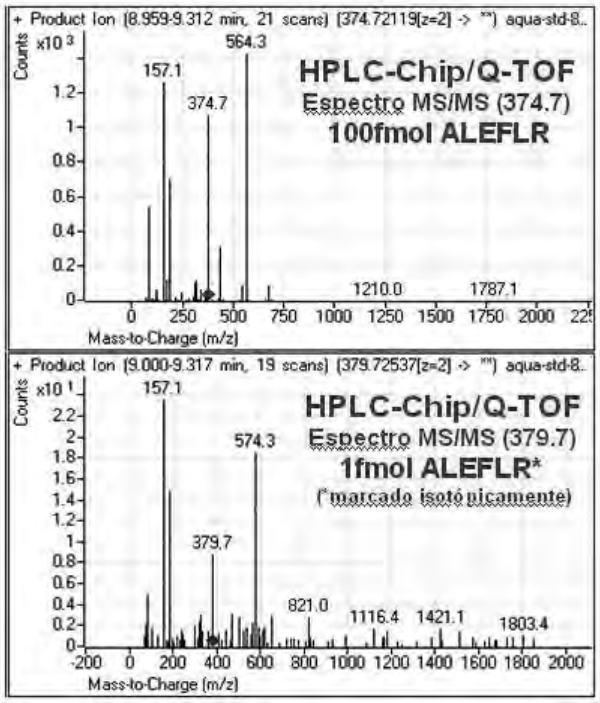

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Quantification of ceramide molecular species in total lipid extracts of white adipose tissue by shotgun lipidomics

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Introduction

Ceramides are low abundant lipids that mediate diverse biological processes such as cell cycle, differentiation and apoptosis. Ceramides usually consist of a long-chain amino alcohol generally referred to as a “sphingoid base” and an amide-linked long-chain fatty acid (Merrill & Sweeley, 1996). ESI/MS has been widely used to directly quantitate molecular species of many classes in lipid extracts of biological samples (Han & Gross, 2003). However, the quantification of low abundant lipids is still a challenge. Herein we present a shotgun lipidomic approach for the characterization and quantification of ceramide lipids in total extracts of white adipose tissue (WAT).

Material and methods

Total lipid extracts were obtained out of 20 mg rat WAT by Folch. Acylglycerides, which constitute more than 90% of the lipid contents of this tissue and interfere with the ESI-MS analysis of the mixture by suppressing the signal, were removed from the total lipid extracts by TLC, and ceramides were extracted from TLC bands by Folch (Fig.A, Inset). Ceramide molecular species precursors were then readily identified in positive ion mode by the 18-carbon sphingosine base specific product ions m/z 252.27, 264.27 and 282.27 (Gu et al., 1997) (Fig.B) on a hybrid QSTAR pulsar *i* instrument equipped with an automated nanoflow ion source NanoMate HD System. Six endogenous

ceramide species (Cer16:0, 18:0, 20:0, 22:0, 24:1 and 24:0) were detected using multiple reactions monitoring (MRM). Absolute quantification was performed by addition of non-naturally occurring Cer 17:0 as internal standard prior to extraction. The method was proven to be linear between 1 and 200 nM.

Results

The total ceramide content in rat WAT was approximately 35 pmol/mg of tissue, from which the most abundant were Cer 24:0 (m/z 650.6), Cer 16:0 (m/z 538.6) and Cer 24:1 (m/z 648.6), representing ca. 38, 28 and 20%, respectively, from the total ceramide content.

Conclusions

This rapid methodology might be applied to the study of the role of ceramides in WAT metabolism, and could have important implications for obesity and type II diabetes research.

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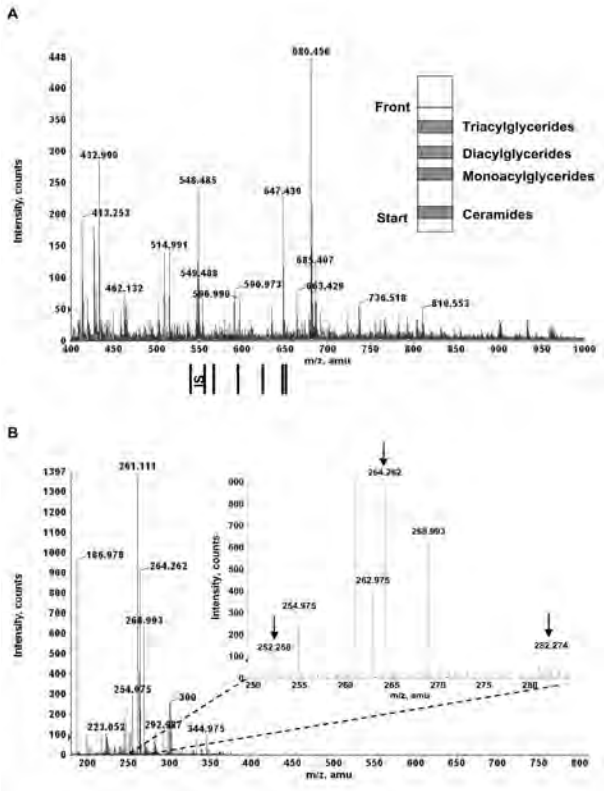

Figure. (A) *Representative survey TOF MS spectrum of a ceramide band obtained from a lipid extract of rat WAT by preparative TLC. The m/z of prospective ceramide precursors are designated by vertical bars. Inset: a schematic TLC run with hexane/etylacetate (5/1, v/v) with assigned bands of various lipid classes.* **(B)** *MS/MS spectrum acquired from the precursor with m/z 538.6 (Cer 16:0). The ceramide-specific fragments m/z 252.27, 264.27, 282.27 used for the quantification are designated with arrows in the inset.*

Análisis diferencial del proteoma de dos cepas de *Aspergillus carbonarius* con diferente nivel de producción de ocratoxina a mediante elecroforesis bidimensional

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Introducción

La ocratoxina A (OTA) es una micotoxina producida por algunas especies de hongos de los géneros *Aspergillus* y *Penicillium*. Dicha micotoxina presenta propiedades carcinogénicas, nefrotóxicas, teratogénicas y neurotóxicas (Bacha et al., 1993). La ingesta más importante de OTA proviene de los